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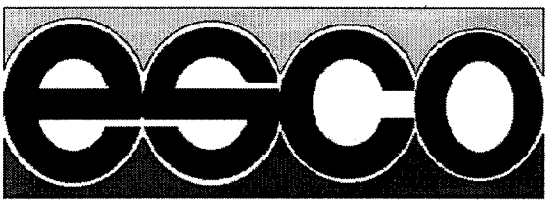


Chemicals for the Information Age

EPA HPV Challenge Color Former Category Robust Summaries - Revised

ESCO Company Limited Partnership
2340 Roberts Street
Muskegon, Michigan 49443

The Color Former Category

Color Former Name	Chemical Name	C.A.S. Number
Black XV	Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'-(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-	36431-22-8
N-102	Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-	29512-49-0
ODB-2	Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-	89331-94-2

Physical and Chemical Elements

1. Melting Point

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity 99.4%

Method

Method	ESCO Company Quality Assurance Laboratory Method
GLP (Yes/No)	No
Year	2002
Remarks	None

Results

Melting Point (°C)	168°C
Decomposition	No
Sublimation	No
Remarks	None

Conclusions

Remarks: The ESCO Company Quality Assurance Laboratory completes melting point testing on each batch of Black XV produced.

Data Quality

Remarks: None

References

None

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 99.5%

Method

Method	ESCO Company Quality Assurance Laboratory Method
GLP (Yes/No)	No
Year	2002
Remarks	None

Results

Melting Point (°C)	195°C
Decomposition	No
Sublimation	No
Remarks	None

Conclusions

Remarks: The ESCO Company Quality Assurance Laboratory completes melting point testing on each batch of N-102 produced.

Data Quality

Remarks: None

References

None

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	EEC Directive 67/548, Annex V, A1 as published in 84/449/EEC
GLP (Yes/No)	Yes
Year	1989
Remarks	None

Results

Melting Point (°C)	181.5 – 185.0°C
Decomposition	No
Sublimation	No
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., “The Determination of the Melting Point/Range of ODB-2,” August 21, 1989.

Other

None

2. Boiling Point

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin), MPBPWIN v1.40

GLP (Yes/No)	No
Year	2003
Remarks	Inputs: Melting point 168°C, Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Results

Boiling Point (°C)	659°C
Decomposition	No
Pressure	Atmospheric
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

ESCO Company ran the MPBPWIN Model on October 20, 2003.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin), MPBPWIN v1.40
GLP (Yes/No)	No
Year	2003
Remarks	Inputs: Melting point 195°C, Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Results

Boiling Point (°C)	636°C
Decomposition	No
Pressure	Atmospheric
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

ESCO Company ran the MPBPWIN Model on October 20, 2003.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin), MPBPWIN v1.40
GLP (Yes/No)	No
Year	2003
Remarks	Inputs: Melting point 182°C, Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Results

Boiling Point (°C)	682°C
Decomposition	No

Pressure	Atmospheric
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

ESCO Company ran the MPBPWIN Model on October 20, 2003.

Other

None

3. Density (Specific Gravity)

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity 99.4%

Method

Method	ESCO Company Quality Assurance Laboratory Method
GLP (Yes/No)	No
Year	1989
Remarks	None

Results

Specific Gravity	1.19 g/mL
Temperature (°C)	20°C
Remarks	None

Conclusions

Remarks: The ESCO Company Quality Assurance Laboratory completed specific gravity determinations on Black XV.

Data Quality

Remarks: None

References

None

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-94-2), Purity 99.5%

Method

Method	ESCO Company Quality Assurance Laboratory Method
GLP (Yes/No)	No
Year	1996
Remarks	None

Results

Specific Gravity	1.19 g/mL
Temperature (°C)	20°C
Remarks	None

Conclusions

Remarks: The ESCO Company Quality Assurance Laboratory completed specific gravity determinations on N-102.

Data Quality

Remarks: None

References

None

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	EEC Directive 67/548, Annex V, A3 as published in 84/449/EEC
GLP (Yes/No)	Yes
Year	1989
Remarks	None

Results

Specific Gravity	1.19 mg/L
Temperature (°C)	20°C
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., “The Determination of the Relative Density of ODB-2,” August 21, 1989.

Other

None

4. Vapor Pressure

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity >99%

Method

Method	EEC Directive 67/548, Annex V, A4 as published in 84/449/EEC
GLP (Yes/No)	Yes
Year	1989
Remarks	None

Results

Vapor Pressure Value	< 2.6 x 10^{-4} Pa at 25°C, 1.0398 x 10^{-3} Pa at 164.25°C 1.1698 x 10^{-3} Pa at 174.25°C, 3.8992 x 10^{-3} Pa at 183.25°C (melting point), 9.618 x 10^{-3} Pa at 194.75°C, and 5.3549 x 10^{-3} Pa at 213°C
Temperature (°C)	At 10 and 20°C above and below its melting point and also at the melting point.
Decomposition	No
Remarks	None

Conclusions

Remarks: “Since no weight displacement was observed below 150°C it can be inferred that the vapor pressure at 25°C is lower than the detection limit of the balance i.e. less than 2.6 x 10^{-4} Pa.”

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., “The Determination of the Vapor Pressure of ODB-2,” August 21, 1989.

Other

None

5. Partition Coefficient

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity >99%

Method

Method	HPLC Method in Annex to Directive 92/69/EEC, Part A, Test A8
GLP (Yes/No)	Yes
Year	1997
Remarks	None

Results

Log P_{ow}	6.5
Temperature °C	21°C
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Life Sciences Ltd., “Black XV Partition Coefficient,” April 2,1997.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 99.8%

Method

Method	HPLC Method in Annex to Directive 92/69/EEC, Part A, Test A8
GLP (Yes/No)	Yes
Year	1997
Remarks	None

Results

Log P_{ow}	>6.2
Temperature °C	20.5°C
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Life Sciences Ltd., “N-102 Partition Coefficient,” April 25,1997.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99%

Method

Method	EEC Directive 67/548, Annex V, A8 as published in 84/449/EEC
GLP (Yes/No)	Yes
Year	1989
Remarks	None

Results

Log P_{ow}	>4.66
Temperature °C	20°C
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Research Centre, Ltd., "The Determination of the Partition Coefficient (n-Octanol/Water) of ODB-2," August 21, 1989.

Other

None

6. Water Solubility

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity >99%

Method

Method	OECD Test Guideline No. 105
GLP (Yes/No)	Yes
Year	1997
Remarks	None

Results

Value (mg/L) at temperature °C	0.0405 mg/L at 25°C
Description of solubility	Not described

pH Value and concentration at temperature °C	7.2 at 0.0405 mg/L at 25°C
pKa Value at 25°C	None provided
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Kurume Research Laboratories, Chemical Biotesting Center, “Measurement of Water Solubility of Black 15 by Column Elution Method,” June 12, 1997

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 99.8%

Method

Method	EEC Directive 92/69/EEC, Part A, Test A6
GLP (Yes/No)	Yes
Year	1997
Remarks	None

Results

Value (mg/L) at temperature °C	0.0202 mg/L at 20°C
Description of solubility	Not described

pH Value and concentration at temperature °C	8.68 at 0.0202 mg/L at 20°C
pKa Value at 25°C	None provided
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Life Sciences Ltd., “N-102 Water Solubility,” June 27, 1997.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99%

Method

Method	EEC Directive 67/548, Annex V, A6 as published in 84/449/EEC
GLP (Yes/No)	Yes
Year	1989
Remarks	None

Results

Value (mg/L) at temperature °C	0.02122 mg/L at 20°C
Description of solubility	Not described

pH Value and concentration at temperature °C	6.12 at 0.02122 mg/L at 20°C
pKa Value at 25°C	See the ODB-2 pKa Value study summary
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., "The Determination of the Water Solubility of ODB-2," August 21, 1989.

Other

None

7. pKa Value

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.79%

Method

Method	OECD Guideline 112
GLP (Yes/No)	Yes
Year	2001
Remarks	None

Results

pKa Value at 25°C	See remarks and conclusion sections

Remarks	The aqueous test solution was insoluble, so an additional amount of tetrahydrofuran solvent was used in an attempt to increase solubility. The extra addition of solvent (to 20%) did not increase ODB-2's solubility in water. An approximation of the pKa value using pure solvent was determined not to be an appropriate comparison to an aqueous solution found in the natural environment.

Conclusions

Remarks: "ODB-2 is not soluble in water even with the addition of a solvent, therefore the OECD 112 Dissociation Guideline Test can not be performed. Dissociation of ODB-2 will not be a significant factor in the natural environment, since it will not dissolve in water at any appreciable levels."

Data Quality

Remarks: None

References

Springborn Laboratories, Inc., "ODB-2 – Determination of the Dissociation Constant," September 6, 2001.

Other

None

8. Adsorption/Desorption to Soil

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 98%

Method

Method	Adsorption/Desorption Batch Equilibrium Method
GLP (Yes/No)	Yes
Year	1993
Remarks	None

Results

Freundlich Adsorption Constant, K_a	The Fruendlich adsorption constants (K_a) ranged from 126 (Somersham) to 2053 (Sandiacre), showing that ODB-2 is strongly adsorbed to the whole range of soils tested.
Remarks	Three soils were used, namely Somersham sandy loam (U.K.), Sandiacre clay loam (U.K.) and Alconbury clay (U.K.) These three soils covered a wide range of organic matter content.

Conclusions

Remarks: "The adsorption/desorption behavior of ODB-2 has been studied in three soils. Based on the results obtained, ODB-2 can be classified as having a low potential for mobility in soil. ODB-2 was very weakly desorbed and had a high affinity for all soil types tested."

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., "Adsorption/Desorption of ^{14}C-ODB-2 with Soil," March 5, 1993.

Other

None

Environmental Fate and Pathway Elements

9. Photodegradation

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	None

Results

Half-life ($t^{1/2}$)	0.05 days
Degradation % after	Not provided
Breakdown products	Not provided
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Conclusions

Remarks: The oxidation program predicts the half-life of Black XV to be very short in air. The AOP program predicts the rate at which the test substance will react with air-borne hydroxyl radicals formed through photochemical reactions. This is the most common type of reaction that organic chemicals undergo in relation to photolysis in air. Although the half-life in air is very short, degradation in air is not expected to be a major degradation pathway since the compound is not particularly volatile.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	None

Results

Half-life ($t^{1/2}$)	0.05 days
Degradation % after	Not provided
Breakdown products	Not provided
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Conclusions

Remarks: The oxidation program predicts the half-life of N-102 to be very short in air. The AOP program predicts the rate at which the test substance will react with air-borne hydroxyl radicals formed through photochemical reactions. This is the most common type of reaction that organic chemicals undergo in relation to photolysis in air. Although the half-life in air is very short, degradation in air is not expected to be a major degradation pathway since the compound is not particularly volatile.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input. The C.A.S. number data base did not include the notation for ODB-2. The SMILES notation for ODB-2 is as follows: O=C4OC5(C6C4=CC=CC=6)C1C(OC3C5=CC=C(N(CCCC)CCCC)C=3)=CC(C)=C(NC2=CC=CC=C2)C=1

Results

Half-life ($t^{1/2}$)	0.05 days
Degradation % after	Not provided
Breakdown products	Not provided
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Conclusions

Remarks: The oxidation program predicts the half-life of ODB-2 to be very short in air. The AOP program predicts the rate at which the test substance will react with air-borne hydroxyl radicals formed through photochemical reactions. This is the most common type of reaction that organic chemicals undergo in relation to photolysis in air. Although the half-life in air is very short, degradation in air is not expected to be a major degradation pathway since the compound is not particularly volatile.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

10. Stability in Water

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Results

Degradation % after	Not provided
Breakdown products	Not provided
Remarks	The model could not calculate water stability data for Black XV since it is not classified as an ester, carbamate epoxide, halmethane, or alkyl halide.

Conclusions

Remarks: The aqueous hydrolysis rate program could not calculate a hydrolytic rate constant for Black XV since it is not classified as an ester, carbamate, epoxide, halmethane, or alkyl halide. Hydrolysis is not likely to be a major pathway for degradation of Black XV since the solubility of Black XV in water is so low.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Results

Degradation % after	Not provided
Breakdown products	Not provided
Remarks	The model could not calculate water stability data for N-102 since it is not classified as an ester, carbamate epoxide, halmethane, or alkyl halide.

Conclusions

Remarks: The aqueous hydrolysis rate program could not calculate a hydrolytic rate constant for N-102 since it is not classified as an ester, carbamate, epoxide, halmethane, or alkyl halide. Hydrolysis is not likely to be a major pathway for degradation of N-102 since the solubility of N-102 in water is so low.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input. The C.A.S. number data base did not include the notation for ODB-2. The SMILES notation for ODB-2 is as follows: O=C4OC5(C6C4=CC=CC=6)C1C(OC3C5=CC=C(N(CCCC)CCCC)C=3)=CC(C)=C(NC2=CC=CC=C2)C=1

Results

Degradation % after	Not provided
Breakdown products	Not provided
Remarks	The model could not calculate water stability data for ODB-2 since it is not classified as an ester, carbamate epoxide, halmethane, or alkyl halide.

Conclusions

Remarks: The aqueous hydrolysis rate program could not calculate a hydrolytic rate constant for ODB-2 since it is not classified as an ester, carbamate, epoxide, halmethane, or alkyl halide. Hydrolysis is not likely to be a major pathway for degradation of ODB-2 since the solubility of ODB-2 in water is so low.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity >99%

Method

Method	Used water stability information included in a "Reproduction test of Black-15 with *Daphnia magna*" to estimate the water stability of Black XV. The test was conducted under OECD Guidelines No. 202
GLP (Yes/No)	Yes
Year	1997
Remarks	The test substance was dissolved in dechlorinated tap water. The concentration of the test substance was measured in the supernatant of the test solution centrifuged. The test substance was measured on day 0, 1, 3, 6, 8, 10, 13, 15, 17, and 20 over a 21-day period. The pH of the test solution ranged from 7.4 to 7.8. The temperature of the test solution ranged from 20.1 to 20.5ºC. The test concentration used was considered to be at saturation in water.

Results

Degradation % after	Not provided
Breakdown products	Not provided
Remarks	Measured Concentrations (mg/l) 0-day 0.0287 mg/l 1-day 0.0320 mg/l 3-day 0.0334 mg/l 6-day 0.0349 mg/l 8-day 0.0251 mg/l 10-day 0.0311 mg/l 13-day 0.0414 mg/l 15-day 0.0370 mg/l 17-day 0.0362 mg/l 20-day 0.0363 mg/l Mean 0.0363 mg/l

Conclusions

Remarks: Based on the measured concentrations of Black XV in water at about 20 ºC and pH at about 7.8, Black XV appears to be in be in equilibrium and does not appear to be breaking down in water. The concentration is not decreasing over time.

Data Quality

Remarks: None

References

Kurume Research Laboratories, Chemical Biotesting Center, Chemicals Inspection and Testing Institute, "Reproduction Test of Black 15 with *Dapnia magna*," December 12, 1997.

11. Transport Between Environmental Compartments (Fugacity)

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin), (Level III Fugacity Model)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input. Physical Property Inputs: Water Solubility: 0.0405 mg/l, Log K_{ow}(octanol /water): 6.50, Melting Point: 168ºC

Results

Media	Air, Water, Soil, and Sediment
Estimated Distribution and Media Concentration	<u>Environmental Distribution</u> Air: 0.01% Water: 1.74% Soil: 36.6% Sediment: 61.7% Persistence: 225 days

	Waste Water Treatment Removal Air: 0.00% Adsorption: 92.65% Biodegradation: 0.78% Total Removal: 93.43% Environmental Half-Life Air: 0.05 days Water: 150.0 days Soil: 150.0 days Sediment: 600.0 days Predicted Parameters Hydrolysis: Can not estimate Atmospheric Oxidation: 35 minutes Biodegradation: Months (recalcitrant) Adsorption: Strong (K_{oc} = 1.1 x 10^8)
Remarks	None

Conclusions

Remarks: Black XV is predicted to bind significantly to soil, sediment, or sludge after entering the natural environment. Black XV is predicted to be persistent in the environment with an overall half-life of almost twenty months.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin), (Level III Fugacity Model)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input. Physical Property Inputs: Water Solubility: 0.0202 mg/l, Log K_{ow}(octanol /water): 6.20, Melting Point: 195ºC

Results

Media	Air, Water, Soil, and Sediment
Estimated Distribution and Media Concentration	<u>Environmental Distribution</u> Air: 0.01% Water: 2.07% Soil: 42.3% Sediment: 55.6% Persistence: 232 days <u>Waste Water Treatment Removal</u> Air: 0.00% Adsorption: 92.07% Biodegradation: 0.77% Total Removal: 92.84% <u>Environmental Half-Life</u> Air: 0.05 days Water: 150.0 days Soil: 150.0 days Sediment: 600.0 days <u>Predicted Parameters</u> Hydrolysis: Can not estimate Atmospheric Oxidation: 35 minutes Biodegradation: Months (recalcitrant) Adsorption: Strong (K_{oc} = 4.15 x 10^7)
Remarks	None

Conclusions

Remarks: N-102 is predicted to bind significantly to soil, sediment, or sludge after entering the natural environment. N-102 is predicted to be persistent in the environment with an overall half-life of almost twenty months.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 100% for model

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin), (Level III Fugacity Model)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input. The C.A.S. number data base did not include the notation for ODB-2. The SMILES notation for ODB-2 is as follows: O=C4OC5(C6C4=CC=CC=6)C1C(OC3C5=CC=C(N(CCCC)CCCC)C=3)=CC(C)=C(NC2=CC=CC=C2)C=1 Physical Property Inputs: Water Solubility: 0.02122 mg/l, Melting Point: 181.5ºC

Results

Media	Air, Water, Soil, and Sediment

Estimated Distribution and Media Concentration	Environmental Distribution Air: 0.02% Water: 2.39% Soil: 28.7% Sediment: 68.9% Persistence: 106 days Waste Water Treatment Removal Air: 0.00% Adsorption: 93.25% Biodegradation: 0.78% Total Removal: 94.03% Environmental Half-Life Air: 0.05 days Water: 60.0 days Soil: 60.0 days Sediment: 240.0 days Predicted Parameters Hydrolysis: Can not estimate Atmospheric Oxidation: 34 minutes Biodegradation: Months (recalcitrant) Adsorption: Strong (K_{oc} = 4.8 x 10^8)
Remarks	None

Conclusions

Remarks: ODB-2 is predicted to bind significantly to soil, sediment, or sludge after entering the natural environment. ODB-2 is predicted to be persistent in the environment with an overall half-life of almost eight months.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

12. Biodegradation

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity 99.1%

Method

Method	OECD Guideline for Testing of Chemicals No. 301D
Test Type	Aerobic
GLP (Yes/No)	Yes
Year	1994
Contact Time	28 days
Innoculum	Activated Sewage Sludge Bacteria
Remarks	There was no evidence of inhibitory effects under the conditions of this test.

Results

Degradation % after time	2% Biodegradation after 28 days
Results	Black XV may not be termed as readily biodegradable.
Kinetic	Sodium benzoate attained 79% biodegradation within 28 days
Breakdown Products	No
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Research Center Ltd., “Black 15 Ready Biodegradability (Closed Bottle test),” June 23, 1994.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 99.4%

Method

Method	OECD Guidelines No. 301B
Test Type	Aerobic
GLP (Yes/No)	Yes
Year	1992
Contact Time	28 days
Innoculum	A mixed population of activated sludge organisms
Remarks	None

Results

Degradation % after time	4% Biodegradation after 28 days
Results	N-102 can not be considered as readily biodegradable.
Kinetic	Sodium benzoate attained 88% degradation after 28 days confirming the suitability of the inoculum and test conditions.
Breakdown Products	No
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Safepharm Laboratories Limited, “Assessment of the Ready Biodegradability (Modified Sturm test) of N-102,” August 25, 1992.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	OECD Guideline No. 301B
Test Type	Aerobic
GLP (Yes/No)	Yes
Year	1992
Contact Time	28 days
Innoculum	Activated sewage sludge
Remarks	None

Results

Degradation % after time	1-2% Biodegradation after 28 days
Results	ODB-2 can not be termed as inherently biodegradable
Kinetic	Sodium benzoate attained 61 % biodegradation within 28 days confirming the suitability of the inoculum and the culture conditions.
Breakdown Products	No
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., “Assessment of the Inherent Biodegradability of ODB-2 (Modified Sturm Test),” January 16, 1992.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity >99%

Method

Method	OECD Guideline No. 301D
Test Type	Aerobic
GLP (Yes/No)	Yes
Year	1989
Contact Time	28 days
Innoculum	Activated sludge bacteria
Remarks	None

Results

Degradation % after time	5% Biodegradation after 28 days
Results	ODB-2 can not be termed as readily biodegradable
Kinetic	Sodium benzoate attained 89 % biodegradation within 28 days. Oxygen depletions in the inoculated and non-inoculated control series were within the prescribed limits.
Breakdown Products	No
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., “Assessment of Ready Biodegradability of ODB-2,” January 6, 1989.

Other

None

Ecotoxicity Elements

13. Acute Toxicity to Fish

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity >99%

Method

Method	OECD Guideline for Testing Chemicals No. 203
Test Type	Acute toxicity to rainbow trout under semi-static conditions.
GLP (Yes/No)	Yes
Year	1998
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Species/Strain/ Supplier	Rainbow trout (*Oncorhynchus mykiss*). Source: Westacre Trout Farm, Norfolk, U.K.
Exposure period	96 hours
Statistical Methods	No mortalities or sublethal effects were recorded during the study (highest test concentration of 7.6 mg/L)
Remarks	None

Results

Nominal Concentrations	10 mg/L
Measured Concentrations	> 7.6 at 3 hours, > 7.6 at 6 hours, > 7.6 at 24 hours, > 7.6 at 48 hours, > 7.6 at 72 hours, > 7.6 at 96 hours
Unit	mg/L
Element Value	96 hour LC_{50} value for Black XV with rainbow trout is > 7.6 mg/L. The “no-observed effect level” is $\geq$ 7.6 mg/L
Statistical Results	Highest test concentration resulting in no mortality: $\geq$ 7.6 mg/L
Remarks	“Near nominal concentrations could not be obtained for the test level in freshly prepared solutions due to the low solubility of Black XV in water (<2.9 mg/L). Further losses over the 24 hour period may have been due to settlement of undissolved test material.”

Conclusions

Remarks: The 96 hour LC_{50} value for Black XV with rainbow trout is > 7.6 mg/L. The "no-observed effect level" is $\geq$ 7.6 mg/L

Data Quality

Remarks: None

References

Huntingdon Life Sciences, Ltd., "Black 15 Acute Toxicity to Rainbow Trout," January 8, 1998.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 99.4%

Method

Method	OECD Guideline for Testing Chemicals No. 203
Test Type	Acute toxicity to Golden orfe under semi-static conditions.
GLP (Yes/No)	Yes
Year	1992
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Species/Strain/ Supplier	Golden orfe (*Leuciscus idus*), Source: Midland Waterlefe, Findern Derby, U.K.
Exposure period	96 hours
Statistical Methods	No mortalities or sublethal effects were recorded during the study (highest test concentration of 10 mg/L)
Remarks	None

Results

Nominal Concentrations	10 mg/L

Measured Concentrations	> 10 at 3 hours, > 10 at 6 hours, > 10 at 24 hours, > 10 at 48 hours, > 10 at 72 hours, > 10 at 96 hours
Unit	mg/L
Element Value	96 hour LC_{50} value for N-102 with golden orfe is > 10 mg/L. The "no-observed effect level" is $\geq$ 10 mg/L
Statistical Results	Highest test concentration resulting in no mortality: $\geq$ 10 mg/L
Remarks	"10 mg/L was the highest test concentration that could be prepared due to the limited solubility of test material in water and auxiliary solvent permitted in the test under the OECD Guidelines."

Conclusions

Remarks: The 96 hour LC_{50} value for N-102 with golden orfe is > 10 mg/L.
The "no-observed effect level" is $\geq$ 10 mg/L.

Data Quality

Remarks: None

References

Safepharm Laboratories Limited, "The Acute Toxicity of N-102 to Golden Orfe," July 24, 1992.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity >99%

Method

Method	OECD Guideline for Testing Chemicals No. 203
Test Type	Acute toxicity to rainbow trout under semi-static conditions.
GLP (Yes/No)	Yes

Year	1998
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Species/Strain/ Supplier	Rainbow trout (*Salmo gairdneri*). Source: Westacre Trout Farm, Norfolk, U.K.
Exposure period	96 hours
Statistical Methods	No mortalities or sublethal effects were recorded during the study (highest test concentration of 1.0 mg/L)
Remarks	None

Results

Nominal Concentrations	1.0 mg/L
Measured Concentrations	> 1.0 at 3 hours, > 1.0 at 6 hours, > 1.0 at 24 hours, > 1.0 at 48 hours, > 1.0 at 72 hours, > 1.0 at 96 hours
Unit	mg/L
Element Value	96 hour LC_{50} value for ODB-2 with rainbow trout is > 1.0 mg/L. The "no-observed effect level" is $\geq$ 1.0 mg/L
Statistical Results	Highest test concentration resulting in no mortality: $\geq$ 1.0 mg/L
Remarks	"1.0 mg/L as the highest test concentration that could be prepared due to the limited solubility of the test material in water and having regard to the amount of auxiliary solvent permitted under OECD Guideline No. 203."

Conclusions

Remarks: The 96 hour LC_{50} value for ODB-2 with rainbow trout is > 1.0 mg/L. The "no-observed effect level" is $\geq$ 1.0 mg/L.

Data Quality

Remarks: None

References

Huntingdon Research Centre, Ltd., "The Acute Toxicity of ODB-2 to Rainbow Trout," April 19, 1989.

Other

None

14. Prolonged Toxicity to Fish

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	OECD Guideline for Testing Chemicals No. 204
Test Type	Prolonged toxicity to rainbow trout under semi-static conditions.
GLP (Yes/No)	Yes
Year	1991
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Species/Strain/ Supplier	Rainbow trout (*Oncorhynchus mykiss*). Source: Westacre Trout Farm, Norfolk, U.K.
Exposure period	21 days
Statistical Methods	No mortalities or sublethal effects were recorded during the study (highest test concentration of 1.0 mg/L)
Remarks	None

Results

Nominal Concentrations	0.010, 0.032, 0.10, 0.32, and 1.0 mg/L
Measured Concentrations	0.0067, 0.020, 0.078, 0.29, and 0.94 mg/L
Unit	mg/L
Element Value	Threshold level of lethal effects: > 0.94 mg/L Threshold level of observed effects: >0.94 mg/L "No observed effect" concentration: $\underline{\geq}$ 0.94 mg/L Threshold LC_{50} concentration: not determined
Remarks	"Higher exposure levels could not be tested due to the limited solubility of the test substance in water and having regard for the limited amount of auxiliary solvent permitted in the test."

Conclusions

Remarks: There was no adverse reactions to exposure with all fish surviving the 21-day test period at the concentrations well in excess of the water solubility value (0.01 mg/L)."

"Length and weight measurements made on all surviving fish at the end of the exposure period indicated that no adverse effects on growth occurred."

Data Quality

Remarks: None

References

Huntingdon Research Centre, Ltd., "The Prolonged Toxicity of ODB-2 to Rainbow Trout," December 20, 1991.

Other

None

15. Bioaccumulation in Rainbow Trout

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	OECD Guideline for Testing Chemicals No. 305E
Test Type	^{14}C-ODB-2 Bioaccumulation in Rainbow Trout
GLP (Yes/No)	Yes
Year	1993
Analytical Monitoring	Thin layer chromatography, Radioactivity was measured by LSC using either Philips Automatic Liquid Scintillation Analyzer or an LKB Analyzer
Species/Strain/ Supplier	Rainbow trout (*Oncorhynchus mykiss*). Source: Westacre Trout Farm, Norfolk, U.K.
Exposure period	28 days
Remarks	Fish were exposed for 28 days to a flow-through system containing ^{14}C-ODB-2 at either the low exposure level (nominally 0.5 µg/L) or the high exposure level (nominally 5.0 µg/L).

Results

Nominal Concentrations	0.5 µg/L and 5.0 µg/L
Results	"1. The bioaccumulation of radioactivity by rainbow trout has been studied during 28 days exposure, under dynamic conditions, to the radiolabelled compound, ^{14}C-ODB-2. Exposure of the ^{14}C-ODB-2 in tank water at two different nominal concentrations of 0.5 and 5.0 µg/L was studied. The elimination of radioactivity has also been studied during depuration period of 14 days. 2. During exposure to a nominal 0.5 and 5 µg/L of ^{14}C-ODB-2, mean concentrations in fish increased to 0.53 and 4.3 µg equiv./g respectively after 3 days and to 2.5 (0.5 µg/L exposure) after 28 days and 15 – 16 (5.0 µg/L exposure) µg equiv./g after 21 – 28 days. The bioconcentration factors after exposure for 28 days were 4300 (0.5 µg/L exposure) and 4800 (5 µg/L exposure). 3. During the 14 days depuration period, mean concentrations in fish had decreased slowly by 40 – 50% to 1.3 (0.5 µg/L exposure) and 9.3 (5.0 µg/L exposure) µg equiv./g. 4. Analysis of the biokinetics of uptake indicate that 95% of the steady state would be reached after 48 days exposure to ^{14}C-ODB-2 at both 0.5 and 5.0 µg/L. Depuration was characterized by elimination half-life of 11 days at both exposure levels."
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Research Centre, Ltd., "^{14}C-ODB-2 Bioaccumulation in Rainbow Trout," June 7, 1993.

Other

None

16. Acute Toxicity to Aquatic Plants (e.g. Algae)

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity >99%

Method

Method	OECD Guideline for Testing Chemicals No. 201
Test Type	Algae growth inhibition test on Black XV
GLP (Yes/No)	Yes
Year	1998
Species/Strain # and Source	*Selenastrum capricornutum*, Strain No.: CCAP 278/4, Source: Cultre Centre of Algae and Protozoa c/o Freshwater Biological Association, Cumbria, U.K.
Element Basis	Cell count/mL, area under the curve, and specific growth rate
Exposure period	72 hours
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Statistical Methods	Logistic regression for the area under the curve and Williams' test for the "No observed effect level"
Remarks	None

Results

Nominal Concentrations	10 mg/L (limit test)
Measured Concentrations	3.1 mg/L
Unit	mg/L
Element Value	E_bC_{50} (72 hours) : > 3.1 mg/L E_rC_{50} (0 – 72 hours): > 3.1 mg/L The "no-observed effect level" is $\geq$ 3.1 mg/L
Statistical Results	No inhibition to growth

Remarks	"All results are based on measured concentrations. Values ranged from 46 - 52% of nominal at 0 hours and 13 - 19% of nominal at 72 hours. The test level of 110 mg/L was used, although it was above the limit of solubility (<2.0 mg/L) and this may explain the low values at 0 hours." "All test and control cultures were inspected microscopically at 72 hours. There were no abnormalities detected in the cells of the control or test cultures." "No cultures showed any signs of contamination by foreign algal cells or protozoa."

Conclusions

Remarks: "Black 15 is not inhibitory to the growth of *Selenastrum capricornutum*, Strain No. CCAP 278/4 at a concentration of 3.1 mg/L. The E_bC_{50} (72 h) and the E_rC_{50} (0 – 72 h) are > 3.1 mg/L."

Data Quality

Remarks: None

References

Huntingdon Life Sciences, Ltd., "Black 15 Algal Growth Inhibition," January 8, 1998.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0) Purity 99.8%

Method

Method	OECD Guideline for Testing Chemicals No. 201
Test Type	Algae growth inhibition test on N-102
GLP (Yes/No)	Yes

Year	1997
Species/Strain # and Source	*Selenastrum capricornutum*, Strain No.: ATCC 22662, Source: American Type Culture Collection, c/o Sales Department, Rockville, Maryland, U.S.A.
Element Basis	Cell count/mL, area under the curve, and specific growth rate
Exposure period	71 hours
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Statistical Methods	Logistic regression for the area under the curve and visual comparison of the measured and calculated growth curves for the "No observed effect level"
Remarks	None

Results

Nominal Concentrations	0, 32, 56, 100 mg/L
Measured Concentrations	Samples not centrifuged at 0 hrs: 0, 9.4, 16.9, 33.7 mg/L Samples not centrifuged at 71 hrs: 0, 8.1, 14.5, 27.1 mg/L Samples centrifuged at 0 hrs: 0, 6.0, 8.9, * mg/L Samples centrifuged at 71 hrs: 0. 3.4, 3.0, 4.7 mg/L * Note: Flask broke during transport, sample lost
Unit	mg/L
Element Value	E_bC_{50} >> 33.7 mg/L E_rC_{50} > 33.7 mg/L The "no-observed effect level" is < 9.0 mg/L
Statistical Results	"The EC_{50} with respect to growth rate and logistic growth (E_rC_{50} was found to be >> 100% (Aqueous suspension). The corresponding E_rC_{10} value was < 32%. The E_bC_{50} value calculated from the area under the growth curve was found to be >> 100% (aqueous suspension). The corresponding E_bC_{10} value was < 32%. The no-observed effect concentration (NOEC) was estimated to be < 32 % of the aqueous suspension."
Remarks	None

Conclusions

Remarks: "Results of the algal growth inhibition test demonstrated some inhibitory effects at measured concentrations far in excess of the stated solubility of N-102 in water. The turbid appearance of the aqueous suspension also indicated that undissolved test substance was present. However, turbidity was

not sufficient to cause growth inhibition due to shading. In such situations it is possible to observe effects due to transfer of test substance from the solid phase to the algal biomass, that may not take place at the solubility level of the test substance."

Data Quality

Remarks: None

References

TNO Nutrition and Food Research Institute, "Effect of N-102 on the Growth of Green Alga," July 14, 1997.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	OECD Guideline for Testing Chemicals No. 201
Test Type	Algae growth inhibition test on ODB-2
GLP (Yes/No)	Yes
Year	1991
Species/Strain # and Source	*Scenedesmus subspicatus*, Strain No.: CCAP 276/20, Source: Cultre Centre of Algae and Protozoa c/o Freshwater Biological Association, Cumbria, U.K.
Element Basis	Cell count/mL, area under the curve, and specific growth rate
Exposure period	72 hours
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Statistical Methods	As outlined in OECD Method No. 201
Remarks	None

Results

Nominal Concentrations	1.0 mg/L

Measured Concentrations	0.76 mg/L
Unit	mg/L
Element Value	E_bC_{50} (72 hours) : > 0.76 mg/L E_rC_{50} (24 - 48 hours): > 0.76 mg/L The "no-observed effect level" is ≥ 0.76 mg/L
Statistical Results	No inhibition to growth
Remarks	"1.0 mg/L (nominal) was the highest test concentration that could be prepared due to the limited solubility of the test substance in water and having regard to the amount of auxiliary solvent permitted in the test. However, because of the unstable nature of the test substance in water under light conditions, the calculated mean measured value of 0.76 mg/L over the study period has been quoted."

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., "The Algistatic Activity of ODB-2," December 6, 1991.

Other

None

17. Acute Toxicity to Aquatic Invertebrates

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity >99%

Method

Method	OECD Guidelines for Testing Chemicals No. 202

Test Type	A 21-day semi-static reproduction test of Black XV with *Daphnia magna* was conducted.
GLP (Yes/No)	Yes
Year	1997
Species/Strain/ Supplier	*Daphnia magna.* Young daphnids produced by parent which cultured in our laboratory were used. They originally came from U.S. E.P.A. Environmental Research Laboratory (Duluth, Minnesota)
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Exposure Period	21 days
Statistical Methods	● Mortality of parent daphnid and time to production of first brood: Nonparametric multiple comparison test (Dunnet's test) followed by rank test (Kruskal-Wallis test) was used for statistical analysis. ● Cumulative number of live offspring per female and test vessel: Parametric multiple comparison test (Dunnet's test) followed by analysis of variance was used for statistical analysis.
Remarks	None

Results

Nominal Concentrations	0.0295 mg/L, 0.0139 mg/L, 0.00717 mg/L (Time-weighted mean measured concentrations)		
Measured Concentrations (mg/L)		0 –1 day	7 – 8 day	14 – 15 day
	HL/4	0.00613	0.0101	0.0102
	HL/2	0.0125	0.0194	0.0205
	HL	0.0287	0.0425	0.0438
Unit	mg/L			
Element Value	No-observed effect concentration: 0.0295 mg/L (time-weighted mean measured value of the dissolved test substance in the highest exposure level)			
Statistical Results	"No observed effect on the survival of parent daphnid, the time to production of first brood, the number of offspring per female, and the conditions of parent daphnid and offspring was observed in the exposure levels compared with controls.			
Remarks	None			

Conclusions

Remarks: "No observed effect on the survival of parent daphnid, the time to production of first brood, the number of offspring per female, and the conditions of parent daphnid and offspring was observed in the exposure levels compared

with controls. The results demonstrate that Black 15 has no adverse effect on daphnid reproduction at the water solubility level."

Data Quality

Remarks: None

References

Kurume Research Laboratories, Chemical Biotesting Center, Chemicals Inspection and Testing Institute, "Reproduction Test of Black 15 with *Dapnia magna*," December 12, 1997.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 99.8%

Method

Method	OECD Guidelines for Testing Chemicals No. 202
Test Type	A 21-day semi-static reproduction test of N-102 with *Daphnia magna* was conducted.
GLP (Yes/No)	Yes
Year	1997
Species/Strain/ Supplier	*Daphnia magna,* Cultured in the laboratory under standard conditions at TNO
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Exposure Period	21 days
Statistical Methods	"Statistical significance for reproduction was determined using the two-tailed Dunnett-test at 95% and 99% significance level using the mean number of young per female as observed values. The observations at each concentration were compared with those of the control. In the case of significance at the 99% level only that significance is given."
Remarks	None

Results

Nominal Concentrations	0%, 32%, 56%, 100% of highest concentration using mechanical stirring which represents the solubility limit of the test substance in the test media.				
Measured Concentrations (mg/L)		5 day	12 day	14 day	19 day
	32%	0.022	0.007	0.031	0.068
	56%	0.026	0.009	0.058	0.086
	100%	0.052	0.010	0.413	0.167
Unit	mg/L				
Element Value	21 day EC50: > aqueous solubility 21 day NOEC: ≥ aqueous solubility 21 day LOEC: > aqueous solubility				
Statistical Results	No observed effect				
Remarks	None				

Conclusions

Remarks: “It is concluded that N-102 is not toxic to Daphnia magna, with respect to reproduction and survival, within the limits of its water solubility when tested as an aqueous extract, prepared by mechanical stirring.”

Data Quality

Remarks: None

References

TNO Nutrition and Food Research Institute, “Semi-static Reproduction Test with N-102 and the Crustacean Species *Daphnia magna*,” July 10, 1997.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	OECD Guidelines for Testing Chemicals No. 202

Test Type	A 21-day semi-static reproduction test of N-102 with *Daphnia magna* was conducted.
GLP (Yes/No)	Yes
Year	1995
Species/Strain/ Supplier	*Daphnia magna,* Cultured in the laboratory under standard conditions at TNO
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Exposure Period	21 days
Statistical Methods	"Statistical significance for mortality was determined with a binomial test at 95% and 99% significance levels combining the results of the quadruplicates. Statistical significance for reproduction was determined using the two-tailed Dunnett-test at 95% and 99% significance levels using the mean number of young per female in each of the four replicates as observed values. In both cases the observations at each concentration were compared with those of the control. In case of significance at the 99% level only that significance is given.
Remarks	None

Results

Nominal Concentrations	Not provided. See remarks section
Measured Concentrations	Not provided. See remarks section
Unit	mg/L
Element Value	Reproduction 21 day EC50 > aqueous solubility 21 day NOEC ≥ aqueous solubility 21 day LOEC > aqueous solubility Survival and Condition 21 day EC50 > aqueous solubility 21 day NOEC ≥ aqueous solubility 21 day LOEC > aqueous solubility
Statistical Results	No observed effect

Remarks	"The chemical analytical results of the daphnia test suffered from a consistently too high detection limit. The calibration standards used in the analysis of the daphnia test samples (0.1 to 2 mg/L) lay outside (above) the range of the samples to be analyzed. The calibration solutions contained from 0.5 to ca. 10% acetonitrile, whereas the injected daphnia test samples contained no organic solvent. Given the hydrophobic nature of the test material, this method was not ideal. The potential discrepancy in the results which can be attributed to this is difficult to determine. A memory effect, attributable to a plastic coupling in the autosampler caused contamination of control samples. Upon checking, this memory effect was apparently reduced by using different model autosampler for additional analyses (not the daphnia test series). It must be assumed that all the daphnia test samples were exposed to a similar type of contamination during the chemical analysis. Given these serious doubts, the authors conclude that the chemical analyses are invalid and can not be used in support of the daphnia reproduction test. They are therefore not presented in this report.

Conclusions

Remarks: "It is concluded that ODB-2 is not toxic to *Daphnia magna*, with respect to reproduction and survival within its aqueous solubility, when tested as an aqueous extract, prepared by mechanical stirring. This is a result which is consistent with what may be expected on basis of the molecule size of ODB-2 (M= 532)."

Data Quality

Remarks: None

References

TNO Nutrition and Food Research Institute, "Semi-static Reproduction Test with ODB-2 and *Daphnia magna*," December 11, 1995.

Other

None

18. Toxicity to Terrestrial Organisms

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	OECD Guideline for Testing Chemicals No. 207
Test Type	Acute toxicity test to earthworm
GLP (Yes/No)	Yes
Year	1991
Species and Source	*Eisenia foetida*, Source: Local Supplier, Houghton, St. Ives, Cambridgeshire, England
Element Basis	Weight loss, and mortality
Exposure period	14 days
Statistical Methods	As outlined in OECD Method No. 207
Remarks	None

Results

Concentrations	0, 95, 171, 309, 556, or 1000 ppm
Unit	ppm
Element Value	"No worms were observed on the soil surface between counts and all surviving worms at Days 7 and 14 appeared normal. No mortalities were detected at the day 7 count. By Day 14, one mortality had occurred in replicate 4C (ODB-2 at 309 ppm); this mortality was not considered to be treatment-related. No mortalities occurred in any other group. Weight losses were observed in all groups, and there was not clear evidence of any treatment-related effect. Because of the limited number of mortalities it was not possible to determine the LC_{50} values of ODB-2 to the earthworm at Days 7 and 14. These values must lie in

	excess of 1000 ppm, the maximum treatment level used."
Statistical Results	Limited number of mortalites
Remarks	"The LC_{50} values for chloroacetamide, tested previously as a positive control between 15 and 29 May 1985 were found to be : Day 7 LC_{50}: 43.1 ppm (95% confidence limits 34.0 – 57.6 ppm) Day 14 LC_{50}: 24.6 ppm (95% confidence limits 20.2 – 30.5 ppm)"

Conclusions

Remarks: "Because of the limited number of mortalities it was not possible to determine the LC_{50} values of ODB-2 to the earthworm at Days 7 and 14. These values must lie in excess of 1000 ppm, the maximum treatment level used."

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., "ODB-2 Acute Toxicity (LC_{50}) to the Earthworm (*Eisenia foetida*)," August 30, 1991.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	OECD Guideline for Testing Chemicals No. 208
Test Type	Higher plant growth study
GLP (Yes/No)	Yes
Year	1993

Species, Variety, and Source	Crop: Wheat; Variety: Alexandria; Source: Twyfords Seeds Crop: Radish; Variety: Shortop Forcing; Source: Suttons Seeds Crop: Lettuce; Variety: Glasshouse Lettuce; Source: Suttons Seeds Crop: Soyabean; Variety: NKS 5960; Source: Herbiseed
Element Basis	Germination counts, plant fresh and dry weights, and crop vigour and phytotoxicity
Exposure period	Through emergence of seedlings and the early stages of growth
Statistical Methods	As outlined in OECD Method No. 208
Remarks	None

Results

Concentrations	0, 1.0, 10, 100 mg/Kg
Unit	mg/Kg

Results

mg ODB-2 per kg of oven dried soil

Final	0	1.0	10	100	Sig. P=0.05
Germination %					
Wheat	100.0	98.3	93.3	96.7	Not sig.
Radish	98.3	98.3	100.0	96.7	Not sig.
Lettuce	100.0	98.0	100.0	102.0	Not sig.
Soyabean	98.3	95.0	100.0	98.3	Not sig.
Fresh Weights, g					
Wheat	2.20	2.28	2.29	2.40	Not sig.
Radish root	5.36	4.82	4.85	5.12	Not sig.
Radish tops	4.63	4.90	4.96	4.98	Not sig.
Lettuce	3.27	3.46	3.33	3.30	Not sig.
Soyabean	2.64	2.50	2.66	2.51	Not sig.
Dry Weights, g					
Wheat	0.32	0.32	0.33	0.35	Not sig.
Radish root	0.28	0.25	0.25	0.26	Not sig.
Radish tops	0.36	0.36	0.37	0.39	Not sig.
Lettuce	0.19	0.20	0.20	0.19	Not sig.
Soyabean	0.39	0.37	0.39	0.38	Not sig.

	Crop Vigour (Scale 1-10) Lettuce 9.17 8.33 9.33 8.67 Not sig. Soyabean 9.50 9.83 9.83 9.33 Not sig.
Statistical Results	No significant differences between treatments were found.
Remarks	"LC_{50} (the concentration at which the change in emergence is 50% of that of control) was not demonstrated by any of the species over the range of concentrations stated." "EC_{50} (the concentration at which the change in growth rate is 50% of that of control) was not demonstrated by any of the species over the range of concentrations stated." "No phytotoxic effects were seen at any time in any of the species during the study. Crop vigour was very good throughout the trial with plants growing uniformly."

Conclusions

Remarks: "No significant differences between treatments were found as assessed by germination counts, plant fresh and dry weights, and crop vigour and phytotoxicity."

Data Quality

Remarks: None

References

Levington Agriculture Ltd., commissioned by Huntingdon Research Centre Ltd. "Higher Plant Growth Studies with ODB-2," June 10, 1993.

Other

None

Health Elements

19. Acute Toxicity

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity: not provided in the study - the estimated purity based on process knowledge is >98%.

Method

Method	OECD Guidelines for Testing Chemicals No. 401
Test Type	Acute Oral Toxicity - Rats
GLP (Yes/No)	Yes
Year	1983
Species/Strain	Sprague-Dawley Rat (albino)
Sex	5 male and 5 female
Number of animals per sex per dose	5 male and 5 female
Vehicle	The sample material was dosed as a 40% w/v suspension in corn oil.
Route of Administration	Each animal was weighed and dosed by direct administration of the experimental material in the stomach by gavage.
Remarks	● Age: No age given, but rats used weighed 180 – 300 grams ● Doses: 10.0 g/kg ● Doses per time period: One dosage level was administered and the rats were allowed food and water ad libitum for the 14 day observation period. ● Volume administered or concentration: 40% w/v in corn oil, 10.0 g/kg. ● Post dose observation period: Observed over 14 days, three times during the first day, and twice daily thereafter with weights recorded at 7 and 14 days.

Results

Value	LD_{50} > 10.0 g/kg
Number of Deaths at each Dose Level	No deaths occurred at the 10.0 g/kg dose level
Remarks	Ave. Prefasted Weight: 224 grams Ave. Prefasted Weight of Survivors: 224 grams Ave. 7-day weight of survivors: 245 grams Ave. 14-day weight of survivors: 255 grams All animals showed expected gain in bodyweight during the study.

Conclusions

Remarks: "The estimated oral rat LD_{50} of test article Black 15 is greater than 10.0 g/kg. Diarrhea occurred in only 2 out of 10 rats. Nothing remarkable observed in the necropsy."

Data Quality

Remarks: None

References

Springborn Group, "Acute Toxicity (LD_{50}) in Rats," December 22, 1983.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity: not provided in the study - the estimated purity based on process knowledge is >98%.

Method

Method	OECD Guidelines for Testing Chemicals No. 401
Test Type	Acute Oral Toxicity - Rats
GLP (Yes/No)	Yes
Year	1993
Species/Strain	Sprague-Dawley Rat
Sex	5 male and 5 female
Number of animals per sex per dose	5 male and 5 female
Vehicle	"For the purpose of this study the test material was freshly prepared, as required, as a suspension at the appropriate concentration in arachis oil B.P."
Route of Administration	"All animals were dosed only once by gavage using a metal cannula attached to a graduated syringe."
Remarks	● Age: Five to eight weeks old, rats used weighed 142 - 169 grams, male; 150 - 163 grams, female ● Doses: 2000 mg/kg ● Doses per time period: One dosage level was

	administered. The rats were freely allowed food and water for the 14 day observation period. ● Volume administered or concentration: 200 mg/ml, Dose volume: 10 ml/kg ● Post dose observation period: Observed over 14 days with deaths and overt signs of toxicity recorded at ½, 1, 2 and 4 hours after dosing and subsequently once daily for 14 days.

Results

Value	LD_{50} > 2000 mg/kg
Number of Deaths at each Dose Level	"There were no deaths. No signs of systemic toxicity were noted during the study."
Remarks	"All animals showed expected gain in bodyweight during the study. No abnormalities were noted at necropsy."

Conclusions

Remarks: "The acute oral median lethal dose (LD_{50}) of the test material in the Sprague-Dawley strain rat was found to be greater than 2000 mg/kg bodyweight."

Data Quality

Remarks: None

References

Safepharm Laboratories Limited, "Acute Oral Toxicity (Limit Test) in the Rat," November 2 , 1993.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity >99%

Method

Method	OECD Guidelines for Testing Chemicals No. 401

Test Type	Acute Oral Toxicity - Rats
GLP (Yes/No)	Yes
Year	1988
Species/Strain	Sprague-Dawley (CD) Rats
Sex	5 male and 5 female
Number of animals per sex per dose	5 male and 5 female
Vehicle	"ODB-2 was prepared at 25% (w/v) concentration in1% aqueous methylcellulose and administered at a volume of 20.0 ml/kg."
Route of Administration	"The appropriate dose volume of the test substance was administered to each rat using a syringe and plastic catheter (8 choke)."
Remarks	● Age: Four to six weeks old, rats used weighed 105 - 128 grams ● Doses: 5.0 g/kg ● Doses per time period: One dosage level was administered. The rats were freely allowed food and water for the 14 day observation period. ● Volume administered or concentration: 20.0 ml/kg ● Post dose observation period: Observed over 14 days – frequently for the remainder of day one after dosing (over a period of five hours) and subsequently twice daily for 14 days.

Results

Value	LD_{50} > 5.0 g/kg bodyweight
Number of Deaths at each Dose Level	"There were no deaths following a single oral dose of ODB-2 at 5.0 g/kg bodyweight."
Remarks	"All rats achieved anticipated bodyweight gains throughout the study. Terminal autopsy findings were normal."

Conclusions

Remarks: "The acute lethal oral dose to rats of ODB-2 was found to be greater than 5.0 g/kg bodyweight."

Data Quality

Remarks: None

References

Huntingdon research Centre Ltd., “Acute Oral Toxicity to Rats of ODB-2,” April 14, 1988.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity: not provided in the study - the estimated purity based on process knowledge is >98%.

Method

Method	OECD Guidelines for Testing Chemicals No. 402
Test Type	Acute Dermal Toxicity - Rats
GLP (Yes/No)	Yes
Year	1990
Species/Strain	Sprague-Dawley Rat
Sex	5 male and 5 female
Number of animals per sex per dose	5 male and 5 female
Vehicle	Undiluted test material, skin moistened with arachis oil B.P.
Route of Administration	“The appropriate amount of test material, as received, was preweighed into a glass vial, and applied uniformly to an area of shorn skin approximating to 10% of the total body surface area which had been previously moistened with arachis oil B.P. A piece of surgical gauze measuring 7 cm x 4 cm was placed over the treatment area and semi-occluded with a piece of self-adhesive bandage (Hypertie).”
Remarks	● Age: Ten to fourteen weeks old, rats used weighed 210 - 227 grams, male; 200 - 207 grams, female ● Doses: 2000 mg/kg ● Doses per time period: One dosage per 24 hour contact time period. After the 24 contact period the bandage was removed and the area wiped with cotton wool moistened with arachis oil B.P. to remove any residual test material.

	● Post dose observation period: Observed over 14 days with deaths and overt signs of toxicity recorded at ½, 1, 2 and 4 hours after dosing and subsequently once daily for 14 days. Individual body weights were recorded on the day of treatment and on days 7 and 14.

Results

Value	LD_{50} > 2000 mg/kg
Number of Deaths at each Dose Level	No deaths occurred at the 2000 mg/kg dose level
Remarks	"There were no deaths. No signs of systemic toxicity or skin irritation were noted during the study. No toxicologically significant effects on bodyweight were noted in the males during the study. One female showed bodyweight loss during the study. No abnormalities were noted at necropsy of animals killed at the end of the study."

Conclusions

Remarks: "The acute dermal median lethal dose (LD_{50}) of the test material in the Sprague-Dawley strain rat was found to be greater than 2000 mg/kg bodyweight."

Data Quality

Remarks: None

References

Safepharm Laboratories Limited, "Black-15: Acute Dermal Toxicity in the Rat," October 24, 1990.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity >99%

Method

Method	OECD Guidelines for Testing Chemicals No. 402
Test Type	Acute Dermal Toxicity - Rats
GLP (Yes/No)	Yes
Year	1988
Species/Strain	Sprague-Dawley (CD) Rat
Sex	5 male and 5 female
Number of animals per sex per dose	5 male and 5 female
Vehicle	Undiluted test material
Route of Administration	"One day prior to treatment hair was removed from the dorso-lumbar region of each rat with electric clippers exposing an area equivalent to 10% of the total body surface. No shaving or chemical depilation was used. The test substance was applied by spreading it evenly over the prepared skin. The treated area (approximately 50 mm x 50 mm) was then promptly covered with gauze which was held in place with an impermeable dressing encircled firmly around the trunk. At the end of the 24-hours exposure period, the dressings were carefully removed and the treated area of skin decontaminated by washing in warm (30º - 40ºC) water and blotting dry with absorbent paper."
Remarks	● Age: Seven to ten weeks old, rats used weighed 205 - 231 grams ● Doses: 2.0 g/kg ● Doses per time period: One dosage per 24 hour contact time period. ● Post dose observation period: Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1. On subsequent days the animals were observed once in the morning and again at the end of the experimental day. Clinical signs were recorded at each observation.

Results

Value	LD_{50} > 2.0 g/kg bodyweight
Number of Deaths at each Dose Level	No deaths occurred at the 2.0 g/kg dose level

Remarks	"There were no clinical signs of systematic reaction to treatment. Application of the test substance caused no irritation reactions or other dermal changes at the treatment sites. A slightly lower bodyweight gain was recorded for one female rat on day 8. Other rats achieved anticipated bodyweight gains throughout the study. No macroscopic abnormalities were found during the autopsy procedure."

Conclusions

Remarks: "The acute lethal dermal dose to rats of ODB-2 was found to be greater than 2.0 g/kg bodyweight."

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., "Acute Dermal Toxicity to Rats of ODB-2," March 15, 1988.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity: not provided in the study - the estimated purity based on process knowledge is >98%.

Method

Method	OECD Guidelines for Testing Chemicals No. 404
Test Type	Primary Skin Irritation - Rabbits
GLP (Yes/No)	Yes
Year	1983
Species/Strain	New Zealand White Rabbits (albino)
Sex	Not specified
Number of animals per sex per dose	3 rabbits

Vehicle	Undiluted test material
Route of Administration	"The hair is removed the day before testing from an area of the back using a small animal clipper. Did not abrade the skin. Apply 0.5 grams of test article under a square surgical gauze patch measuring 1 inch x 1 inch and two-ply thick to the test site. Secure the patches in place with adhesive tape by placing tape around the border of the patch. Semi-occlude the patch with rubber dental dam and wrap the edges with elastic tape. After 4 hours, remove the restrainer and wraps. Wipe off excess test material wash only if test article residue obscures the sites from evaluation."
Remarks	● Age: no age given, rabbits used weighed $\geq$ 2.0 kg ● Doses: 0.5 grams ● Doses per time period: One dosage per 4 hour contact time period. ● Post dose observation period: Evaluated the test sites once per day for 3 days. Evaluated for corrosion, erythema, and edema

Results

Value	Primary Irritation Index: 0.0
Remarks	None

Conclusions

Remarks: "Test article Black 15 is considered to be not corrosive to skin of rabbits and produced no observable skin irritation. The primary irritation index was 0.0."

Data Quality

Remarks: None

References

Springborn Institute for Bioresearch, Inc., "Black-15: Primary Skin Irritation/Corrosion," December 22, 1983.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 99.8%

Method

Method	OECD Guidelines for Testing Chemicals No. 404
Test Type	Primary Skin Irritation - Rabbits
GLP (Yes/No)	Yes
Year	1997
Species/Strain	New Zealand White Rabbits (albino)
Sex	Male
Number of animals per sex per dose	3 rabbits
Vehicle	Undiluted test material
Route of Administration	"Approximately 24 hours prior to application of the test substance, hair was removed with electric clippers from the dorso-lumbar region of each rabbit exposing an area of skin approximately 100 mm x 100 mm. Approximately 0.5 grams of the test substance was applied under a 25 mm x 25 mm gauze pad which had been moistened with 0.5 grams distilled water to one intact skin site on each animal. Each treatment site was covered with 'Elastoplast' elastic adhesive dressing for four hours. The animals were not restrained during the exposure period and were returned to their cages immediately after treatment. At the end of the exposure period, the semi-occlusive dressing and gauze pad were removed and the treatment site was washed with warm water (30º to 40ºC) to remove any residual test substance. The treated area was blotted dry with absorbent paper."
Remarks	● Age: Ten to eleven weeks, rabbits used weighed 2.2 to 2.5 kg ● Doses: 0.5 grams ● Doses per time period: One dosage per 4 hour contact time period. ● Post dose observation period: Examination of the treated skin was made on Day 1 (i.e. approximately 30 minutes after removal of the patches) and on Days 2,3, and 4." Evaluated for erythema, and edema

Results

Value	"No dermal reactions were observed in any animal throughout the study."
Remarks	The numerical values were all "0." "There were no signs of toxicity or ill health in any rabbit during the observation period."

Conclusions

Remarks: "A single semi-occlusive application of N-102 to intact rabbit skin for four hours elicited no dermal irritation."

Data Quality

Remarks: None

References

Huntingdon Life Sciences Ltd., "N-102: Skin Irritation to the Rabbit," February 13, 1997.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity >99%

Method

Method	OECD Guidelines for Testing Chemicals No. 404
Test Type	Primary Skin Irritation - Rabbits
GLP (Yes/No)	Yes
Year	1988
Species/Strain	New Zealand White Rabbits (albino)
Sex	Male
Number of animals per sex per dose	3 rabbits - 2 male, 1 female
Vehicle	Undiluted test material

Route of Administration	"Approximately 24 hours prior to application of the test substance, hair was removed with electric clippers from the dorso-lumbar region of each rabbit exposing an area of skin approximately 10 cm square. Approximately 0.5 grams of the test substance was applied under a 2.5 cm square gauze pad moistened with 0.5 ml distilled water to one intact skin site on each animal. Each treatment site was occluded with 'Elastoplast' elastic adhesive dressing for a four hour period. The animals were not restrained during the exposure period and were returned to their cages. At the end of the exposure period, the semi-occlusive dressing and gauze pad were removed and the treatment site was washed using water to remove any residual test substance."
Remarks	● Age: Eleven to fourteen weeks, rabbits used weighed 2.5 to 3.2 kg ● Doses: 0.5 grams ● Doses per time period: One dosage per 4 hour contact time period. ● Post dose observation period: "Examination of the treated skin was made on Day 1 (i.e. approximately 30 minutes after removal of the patches) and on Days 2,3, and 4." Evaluated for erythema, and edema.

Results

Value	"None of the animals showed any response to treatment."
Remarks	The numerical values were all "0." "There were no signs of toxicity or ill health in any rabbit during the observation period."

Conclusions

Remarks: "A single semi-occlusive application of ODB-2 to intact rabbit skin for four hours elicited no dermal irritation."

Data Quality

Remarks: None

References

Huntingdon Life Sciences Ltd., "Irritant Effects on Rabbit Skin of ODB-2," March 21, 1988.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity: not provided in the study - the estimated purity based on process knowledge is >98%.

Method

Method	OECD Guidelines for Testing Chemicals No. 405
Test Type	Acute Eye Irritation - Rabbits
GLP (Yes/No)	Yes
Year	1989
Species/Strain	New Zealand White Rabbits
Sex	2 male, 1 female
Number of animals per sex per dose	3 rabbits – 2 male, 1 female
Vehicle	Undiluted test material
Route of Administration	"Immediately before commencement of the test, both eyes of the three provisionally selected test rabbits were examined for evidence of ocular irritation or defect using the light source from a standard opthalmoscope. Animals showing evidence of ocular lesions were rejected and replaced. On the day of the test each animal was held firmly but gently until quiet. A volume of 0.1 ml of the test material (as measured by gently compacting the required volume into an adapted syringe) which was found to weigh approximately 40 mg was placed into the right eye of each rabbit by gently pulling the lower lid away from the eyeball to form a cup into which the test material was dropped. The upper and lower eyelids were held together for about one second immediately after application, to prevent loss of the test material, and then released. The left eye remained untreated and was used for control purposes."

Remarks	● Age: Twelve to sixteen weeks old, rabbits used weighed 2.2 – 2.66 kg ● Doses: 0.1 ml (40 mg) ● Doses per time period: One dosage per 72 hour observation period. ● Post dose observation period: Assessment of damage/irritation was made 1, 24, 48, and 72 hours following treatment.

Results

Value	"The test material produced a maximum group mean score of 15.7 and was classified as a mild irritant (Class 4 on a 1 to 8 scale) to the rabbit eye according to a modified Kay and Calandra scoring system."
Remarks	"A dulling of the normal lustre of the corneal surface was noted in two treated eyes one hour after treatment and in one treated eye at the 24-hour observation. No other adverse corneal effects were noted. Iridial inflammation was noted in all treated eyes one hour after treatment. No other adverse effects were noted. Moderate conjunctival irritation (redness grade 2, swelling and discharge grades 1-2) were noted in all treated eyes one hour after treatment. Minimal conjunctival irritation (redness grade 2) persisted in two treated eyes at the 24-hour observation. The remaining treated eyes appeared normal at subsequent 48 and 72-hour observations."

Conclusions

Remarks: "The test material, Black-15, was found to be a mild irritant to the rabbit eye."

Data Quality

Remarks: None

References

Safepharm Laboratories Limited, "Black-15: Acute Eye Irritation test in the Rabbit," December 22, 1988.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 99.8%

Method

Method	OECD Guidelines for Testing Chemicals No. 405
Test Type	Acute Eye Irritation - Rabbits
GLP (Yes/No)	Yes
Year	1997
Species/Strain	New Zealand White Rabbits
Sex	Male
Number of animals per sex per dose	3 rabbits – 3 male
Vehicle	Undiluted test material
Route of Administration	"The eyes of each animal were examined prior to instillation of the test substance to ensure that there was no pre-existing corneal damage, iridial or conjunctival inflammation. One animal was treated in advance of the others, to ensure that if a severe response was produced, no further animals would be exposed. Approximately 70 mg of the test substance, the weight occupying a volume of 0.1 ml, was placed into the lower everted lid of one eye of each animal. The eyelids were then gently held together for one second before releasing. The contralateral eye remained untreated."
Remarks	● Age: Thirteen to sixteen weeks old, rabbits used weighed 3.0 - 3.6 kg ● Doses: 0.1 ml (70 mg) ● Doses per time period: One dosage per 72 hour observation period. ● Post dose observation period: Examination of the eyes was made after 1 hour and 1,2,3 days (equivalent to 24, 48, and 72 hours) after instillation. Additional observations were made for two animals four days after treatment. Observation of the eyes was aided by the use of a handheld light. Ocular irritation was assessed on the cornea, iris, conjunctivae, and chemosis.

Results

Value	"There were no signs of toxicity or ill health in any rabbit during the observation period." "No corneal damage or iridial inflammation was observed. Transient hyperemia of blood vessels to a diffuse crimson coloration of the conjunctivae with slight swelling or partial eversion of the eyelids was observed in all animals. These responses had resolved completely by two days after instillation."
Remarks	None

Conclusions

Remarks: "Instillation of N-102 into the rabbit eye elicited transient very slight to well defined conjunctival irritation only."

Data Quality

Remarks: None

References

Huntingdon Life Sciences Ltd., "N-102: Eye Irritation to the Rabbit," March 7, 1997.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity >99%

Method

Method	OECD Guidelines for Testing Chemicals No. 405
Test Type	Acute Eye Irritation - Rabbits
GLP (Yes/No)	Yes
Year	1997

Species/Strain	New Zealand White Rabbits
Sex	Male
Number of animals per sex per dose	3 rabbits – 3 female
Vehicle	Undiluted test material
Route of Administration	"The eyes of each animal were examined prior to instillation of the test substance to ensure that there was no pre-existing corneal damage, iridial or conjunctival inflammation. A 40 mg amount of ODB-2, the weight occupying a volume of 0.1 ml, was placed into the lower everted lid of one eye of each animal. The eyelids were then gently held together for one second before releasing. The contralateral eye remained untreated and served as a control."
Remarks	● Age: Twelve to sixteen weeks old, rabbits used weighed 2.9 - 3.7 kg ● Doses: 0.1 ml (40 mg) ● Doses per time period: One dosage per 72 hour observation period. ● Post dose observation period: Examination of the eyes was made after 1 hour and 1,2,3 and 4 days after instillation. Observation of the eyes was aided by the use of a handheld light. Ocular irritation was assessed on the cornea, iris, and conjunctivae.

Results

Value	"None of the animals gave a positive response. No corneal damage or iridial inflammation was observed. Very slight conjuctival irritation was observed in all three animals at the one hour reading only. All eyes were completely normal the day after instillation of the test substance."
Remarks	None

Conclusions

Remarks: "Instillation of ODB-2 into the rabbit eye did not elicit a positive response in any of the three treated animals according to OECD test criteria."

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., "Irritant Effects on the Rabbit Eye to ODB-2," April 28, 1988.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity: not provided in the study - the estimated purity based on process knowledge is >98%.

Method

Method	Maximization Technique (Magnusson and Kligman), satisfies OECD Guidelines
Test Type	Delayed Contact Hypersensitivity Study – Guinea Pigs
GLP (Yes/No)	No
Year	1993
Species/Strain	Hartley albino guinea pigs
Sex	28 male, 27 female
Number of animals per sex per dose	55 Guinea pigs – 28 male, 27 female
Vehicle	Polyethylene glycol 400
Route of Administration	Primary Irritation: 0.5%, 1%, 2.5%, 5%, 10%, 25%, 50% Black XV in Polyethylene glycol 400 at 0.1 ml Injection Induction: 5% w/v Black XV in Polyethylene glycol 400 at 0.1 ml Topical Induction: 50% w/v Black XV in Polyetheylene glycol 400 at 0.8 ml Primary Challenge: 5% w/v Black XV in Polyethylene glycol 400 at 0.4 ml
Remarks	● Age: Not given, Guinea pigs used weighed 447 – 717 grams ● Doses: Primary Irritation: 0.1 ml, Injection: 0.1 ml, Topical: 0.4 ml, Primary: 0.8 ml ● Doses per time period: One dosage per 24 to 48 hour observation period.

	● Post dose observation period: Assessment of damage/irritation was made 2, 24, 48 hours following treatment.

Results

Value	"Following primary challenge, the incidence of grade 1 responses in the test, the vehicle control, the naive test, an naive vehicle control groups was 0 of 25, 0 of 10, and 0 of 10, respectively. The relative incidence of these responses resulted in a classification of weak sensitization for the test material and weak sensitization for the vehicle control material."
Remarks	"Note: Classification in accordance with the protocol categorizes the test material and the vehicle control material at the primary challenge as causing a weak rate of sensitization response. It is important to note that this category includes a 0% sensitization rate."

Conclusions

Remarks: "The relative incidence of these responses resulted in a classification of weak sensitization for the test material and weak sensitization for the vehicle control material."

Data Quality

Remarks: None

References

Hilltop Biolabs, Inc., "Delayed Contact Hypersensitivity Study in Guinea Pigs of Black XV," January 21, 1993.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity: not provided in the study - the estimated purity based on process knowledge is >98%.

Method

Method	Maximization Technique (Magnusson and Kligman), satisfies OECD Guidelines
Test Type	Delayed Contact Hypersensitivity Study – Guinea Pigs
GLP (Yes/No)	Yes
Year	1996
Species/Strain	Hartley albino guinea pigs
Sex	Male and female
Number of animals per sex per dose	75 Guinea pigs – 38 male, 37 female
Vehicle	Acetone
Route of Administration	Primary Irritation: 0.25%, 0.5%, 1%, 2.5%, 5%, 10%, 25%, 50% N-102 in acetone at 0.1 ml Injection Induction: 5% w/v N-102 in acetone at 0.1 ml Topical Induction: 50% w/v N-102 in acetone at 0.8 ml Primary Challenge: 50% w/v N-102 in acetone at 0.4 ml
Remarks	● Age: Young adult, Guinea pigs used weighed 353 – 569 grams ● Doses: Primary Irritation: 0.1 ml, Injection: 0.1 ml, Topical: 0.4 ml, Primary: 0.8 ml ● Doses per time period: One dosage per 24 to 48 hour observation period. ● Post dose observation period: Assessment of damage/irritation was made 24 and 48 hours following treatment.

Results

Value	"Following primary challenge using N-102 as a 50% w/v formulation in acetone, the incidence of grade 1 responses in the test, the vehicle control, and the naive control groups was 0 of 25, 0 of 10, and 0 of 10, respectively. Following concurrent primary challenge using undiluted acetone, the incidence grade of 1 responses in the test, the vehicle control, and the naive control groups was 0 of 25, 0 of 10, and 0 of 10, respectively."

Remarks	"For validation of the test system, a positive control group was evaluated concurrently with the test group. Following primary challenge using α-hexylcinnamaldehyde, tech 85% as a 5% w/v formulation in acetone, the incidence of grade 1 responses or greater in the positive control group and the naive positive control group was 5 of 10 and 0 of 10 respectively."

Conclusions

Remarks: "Under EEC Guidelines, the 0% incidence in grade 1 responses to N-102 as a 50% w/v formulation in acetone in the test group at challenge, relative to that of the appropriate controls, indicates a nonsensitizer for European labeling purposes. In addition, the 0% incidence of grade 1 responses to the acetone vehicle control material in the vehicle control group at challenge, relative to that of the appropriate controls, also indicates a nonsensitizer for these labeling purposes."

"Under TSCA Guidelines, the incidence of the responses in each group at challenge, relative to the appropriate controls, as described above, indicates a 0% sensitization rate for N-102 as a 50% w/v formulation in acetone and a 0% sensitization rate for the acetone vehicle control material. This corresponds into a classification of 'weak sensitization' for N-102 and 'weak sensitization' for the acetone vehicle control material. It is important to note that the category, 'weak sensitization,' includes a 0% sensitization rate."

Data Quality

Remarks: None

References

Hill Top Research, Inc., "Delayed Contact Hypersensitivity Study in Guinea Pigs of N-102," October 17, 1996.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity: not provided in the study - the estimated purity based on process knowledge is >98%.

Method

Method	Maximization Technique (Magnusson and Kligman), satisfies OECD Guidelines
Test Type	Delayed Contact Hypersensitivity Study – Guinea Pigs
GLP (Yes/No)	Yes
Year	1996
Species/Strain	Hartley albino guinea pigs
Sex	Male and female
Number of animals per sex per dose	75 Guinea pigs – 37 male, 38 female
Vehicle	Acetone
Route of Administration	Primary Irritation: 0.25%, 0.5%, 1%, 2.5%, 5%, 10%, 25%, 50% N-102 in acetone at 0.1 ml Injection Induction: 2.5% w/v N-102 in acetone at 0.1 ml Topical Induction: 50% w/v N-102 in acetone at 0.8 ml Primary Challenge: 50% w/v N-102 in acetone at 0.4 ml
Remarks	● Age: Young adult, Guinea pigs used weighed 353 – 569 grams ● Doses: Primary Irritation: 0.1 ml, Injection: 0.1 ml, Topical: 0.4 ml, Primary: 0.8 ml ● Doses per time period: One dosage per 24 to 48 hour observation period. ● Post dose observation period: Assessment of damage/irritation was made 24 and 48 hours following treatment.

Results

Value	"Following primary challenge using ODB-2 as a 50% w/v formulation in acetone, the incidence of grade 1 responses in the test, the vehicle control, and the naive control groups was 0 of 25, 0 of 10, and 0 of 10, respectively. Following concurrent primary challenge using undiluted acetone, the incidence grade of 1 responses in the test, the vehicle control, and the naive control groups was 0 of 25, 0 of 10, and 0 of 10, respectively."

Remarks	"For validation of the test system, a positive control group was evaluated concurrently with the test group. Following primary challenge using α-hexylcinnamaldehyde, tech 85% as a 5% w/v formulation in acetone, the incidence of grade 1 responses or greater in the positive control group and the naive positive control group was 5 of 10 and 0 of 10 respectively."

Conclusions

Remarks: "Under EEC Guidelines, the 0% incidence in grade 1 responses to ODB-2 as a 50% w/v formulation in acetone in the test group at challenge, relative to that of the appropriate controls, indicates a nonsensitizer for European labeling purposes. In addition, the 0% incidence of grade 1 responses to the acetone vehicle control material in the vehicle control group at challenge, relative to that of the appropriate controls, also indicates a nonsensitizer for these labeling purposes."

"Under TSCA Guidelines, the incidence of the responses in each group at challenge, relative to the appropriate controls, as described above, indicates a 0% sensitization rate for ODB-2 as a 50% w/v formulation in acetone and a 0% sensitization rate for the acetone vehicle control material. This corresponds into a classification of 'weak sensitization' for ODB-2 and 'weak sensitization' for the acetone vehicle control material. It is important to note that the category, 'weak sensitization,' includes a 0% sensitization rate."

Data Quality

Remarks: None

References

Hill Top Research, Inc., "Delayed Contact Hypersensitivity Study in Guinea Pigs of ODB-2," October 17, 1996.

Other

None

Genetic Toxicity Elements

20. Genetic Toxicity In Vitro

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity: not provided in the study - the estimated purity based on process knowledge is >98%.

Method

Method	Microbiological Associates, Inc., Chromosome Aberration Method (Evans 1976)
Test Type	Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells (Cytogenetic assay)
System of Testing	Chinese Hamster Ovaries
GLP (Yes/No)	Yes
Year	1990
Species/Strain	Chinese Hamster Ovary (CHO-K_1) cells (Repository Number CCL 61, American Type Culture Collection, Rockville, Maryland)
Metobolic Activation	Species and cell type: Aroclor 1254 – induced rat liver S-9 (male Sprague-Dawley rat) Quantity: 5 ml Induced or not induced: Studied induced and non-induced cells
Concentrations Tested	37.5, 75, 150, and 300 µg/ml
Statistical Methods	"Statistical analysis of the percent aberrant cells was performed using Fisher's exact test. The Fisher's test was used to compare pairwise the percent aberrant cells of each treatment group with that of solvent control. In the event of positive Fisher's exact test at any test article dose level, the Conchran-Armitage test was used to measure dose-responsiveness."
Remarks	● Culture Harvest Time: 10 hours ● Metaphases Examined: "CHO cells were seeded at approximately 5 x 10^5 cells/25 cm^2 flask. Treatment was carried out by refeeding duplicate flasks with 5 ml complete medium for the nonactivated study and 5 ml S-9 reaction mixture for the activated study, to which was added 50 µl of dosing solution of test or control article in solvent or solvent alone." The test article solvent vehicle was dimethyl sulfoxide (DMSO). ● Cytotoxic Concentration: >300µg/l, the maximum concentration tested

	● Precipitation: “The test article slightly clouded the treatment medium at test concentration 150 µg/ml and precipitated upon addition to treatment medium at dose level 300 µg/ml.” ● Positive Controls: “Triethyleneamine was used as the positive control in the non-activated study at a final concentration of 0.5 µg/l. Cyclophosphamide was use as the positive control in the S-9 activated study at a final concentration of 50 µg/l.” ● Criteria for Determining Positive Results: “The toxic effects of treatment are based upon mitotic inhibition relative to the solvent-treated control and are presented for the toxicity and aberration study. The number and types of aberrations found are presented for each treatment group. The percentage of structurally damaged cells (percent aberrant cells) in the total population of cells examined was calculated for each group. The frequency of structural aberrations per cell (mean aberrations per cell) was also calculated and reported for each group. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell.” “The test article was considered to induce a positive response when the percentage of cells with aberrations are increased in a dose-response manner with one or more concentrations being statistically elevated relative to the solvent control group ($p \leq 0.05$). A significant increase at the high dose only with no dose response was considered suspect. A significant increase at one dose level other than the high dose with no dose response was considered equivocal.”

Results

Statistical Results	“The percentage of cells with structural aberrations in the test article-treated groups was not significantly increased above that of the solvent control.” This results was true for the metabolic activated and the non-activated groups.”
Remarks	With metabolic activation: no test concentration caused aberrations Without metabolic activation: no test concentration caused aberrations

Conclusions

Remarks: “The positive and negative controls fulfilled the requirements for a valid test. Under the conditions of the assay described in this report, Black XV was concluded to be negative in the CHO cytogenetics assay.”

Data Quality

Remarks: None

References

Microbiological Associates, Inc., “Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells,” November 9, 1990.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity: 99.5%

Method

Method	Microbiological Associates, Inc., Chromosome Aberration Method (Evans 1976)
Test Type	Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells (Cytogenetic assay)
System of Testing	Chinese Hamster Ovaries
GLP (Yes/No)	Yes
Year	1997
Species/Strain	Chinese Hamster Ovary (CHO-K_1) cells (Repository Number CCL 61, American Type Culture Collection, Rockville, Maryland)
Metobolic Activation	Species and cell type: Aroclor 1254 – induced rat liver S-9 (male Sprague-Dawley rat) Quantity: 1 ml S-9 in 4 ml of serum-free medium Induced or not induced: Studied induced and non-induced cells
Concentrations Tested	3.8, 11.4,38, 114, 380, 400, 600, 800, 1050, 1100, 1130, and 3760 µg/ml

Statistical Methods	"Statistical analysis of the percent aberrant cells was performed using Fisher's exact test. The Fisher's test was used to compare pairwise the percent aberrant cells of each treatment group with that of solvent control. In the event of positive Fisher's exact test at any test article dose level, the Conchran-Armitage test was used to measure dose-responsiveness."
Remarks	● Culture Harvest Time: "In the nonactivated portion of the independent repeat assay, CHO cells were exposed to the test article for 20 and 44 hours continuously; in the S-9 activated portion of the assay, the cells were exposed to the test article for 6 hours." ● Metaphases Examined: "The chromosome aberration assay was performed using standard procedures by exposing duplicate cultures of CHO cells to the test article as well as positive, solvent, and untreated controls. CHO cells were seeded at approximately 5 x 10^5 cells/25 cm^2 flask for cell collection times of 20 hours and at approximately 3 x 10^5 cells/25 cm^2 flask for cell collection times in excess of 20 hours. Treatment was carried out by refeeding duplicate flasks with 5 ml complete medium for the nonactivated study and 4 ml serum-free dosing solution and 1 ml S-9 reaction mixture for the activated study, to which was added 50 µl of dosing solution of test or control article in solvent or solvent alone." The test article solvent vehicle was dimethyl sulfoxide (DMSO). ● Cytotoxic Concentration: >3,760µg/l, the maximum concentration tested ● Precipitation: "The test article was a workable suspension in treatment medium at concentrations of 380, 1130 and 3760 µg/l. The treatment medium was soluble but cloudy at a concentration of 114 µg/l. Concentrations of $\leq$ 38 µg/l were soluble in treatment medium." ● Positive Controls: "Mitomycin C was used as the concurrent positive control in the non-activated for the initial and independent repeat cytogenetic assays. Cyclophosphamide was used as the positive control in the S-9 activated study for the initial and independent repeat cytogenetic assays at a final concentration of 10 µg/l."

	● Criteria for Determining Positive Results: "The toxic effects of treatment are based upon cell growth inhibition relative to the solvent-treated control and are presented for both initial and the independent repeat assay. The number and types of structural chromosome aberrations, the percentage of cells with structural chromosome aberrations (percent aberrant cells) in the total population of cells examined, the overall structural chromosome aberration frequency, and the mean structural chromosome aberrations per cell was calculated and reported for each group. The number of polyploid and endoreduplicated cells will also be reported and the total percentage of cells with numerical aberrations will be calculated. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell." "The test article was considered to induce a positive response when the percentage of cells with aberrations are increased in a dose-response manner with one or more concentrations being statistically elevated relative to the solvent control group ($p \leq 0.05$). A reproducible and significant increase at a single dose level also will be considered positive. Test articles not demonstrating a statistically significant increase in aberrations will be concluded to be negative."

Results

Statistical Results	"Non statistically significant increases in structural chromosome aberrations were observed in either the non-activated or S-9 activated studies, regardless of dose level or harvest time ($p>0.05$, Fisher's exact test). No Statistically significant increases in numerical chromosome aberrations were observed in either the non-activated of S-9 activated studies at the 44 hour harvest time, at any dose level ($p>0.05$, Fisher's exact test)."
Remarks	With metabolic activation: no test concentration caused chromosome aberrations Without metabolic activation: Substantial toxicity ($\geq$ 50% cell growth inhibition) was observed at the highest dose level evaluated for chromosome aberrations, 1050 µg/ml, in the non-activated 20 hour continuous exposure; at 1130 µg/ml, in the non-activated 44 hour continuous exposure.

Conclusions

Remarks: "All criteria for a valid study were met as described in the protocol. Under the conditions of this study, test article N102 was concluded to be negative for the induction of structural and numerical chromosome aberrations in the *in vitro* mammalian cytogenetics test."

Data Quality

Remarks: None

References

Microbiological Associates, Inc., "*In Vitro* Mammalian Cytogenetic Test Using Chinese Hamster Ovary (CHO) Cells," July 10, 1997.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity >99%

Method

Method	OECD Guidelines for Testing Chemicals No. 473
Test Type	Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells (Cytogenetic assay)
System of Testing	Chinese Hamster Ovaries
GLP (Yes/No)	Yes
Year	1989
Species/Strain	Chinese Hamster Ovary (CHO) Cells, Strain: K_1-BH_4, Obtained from BIBRA
Metobolic Activation	Species and cell type: Aroclor 1254 – induced rat liver S-9 (male Sprague-Dawley rat) Quantity: 1.25 µl S-9 Induced or not induced: Studied induced and non-induced cells
Concentrations Tested	4, 20 and 40 µg/ml

Statistical Methods	Fisher's exact test
Remarks	● Culture Harvest Time: In the nonactivated portion of the independent repeat assay, CHO cells were exposed to the test article for 21 hours; in the S-9 activated portion of the assay, the cells were exposed to the test article for 4 hours. ● Metaphases Examined: "The chromosome aberration assay was performed using standard procedures by exposing duplicate cultures of CHO cells to the test article as well as positive, solvent, and untreated controls. CHO cells were seeded at approximately 8 x 10^4 cells/ml. The test article solvent vehicle was dimethyl sulfoxide (DMSO). ● Cytotoxic Concentration: >40 µg/l, the maximum concentration tested ● Precipitation: No precipitation was observed. ● Positive Controls: Mitomycin C was used as the positive control in the non-activated assays at a final concentration of 0.4 µg/l. Cyclophosphamide was used as the positive control in the S-9 activated assays at a final concentration of 20 µg/l." ● Criteria for Determining Positive Results: The toxic effects of treatment are based upon cell growth inhibition relative to the solvent-treated control and are presented for both initial and the independent repeat assay. The number and types of structural chromosome aberrations, the percentage of cells with structural chromosome aberrations (percent aberrant cells) in the total population of cells examined, the overall structural chromosome aberration frequency, and the mean structural chromosome aberrations per cell was calculated and reported for each group. The number of polyploid and endoreduplicated cells will also be reported and the total percentage of cells with numerical aberrations will be calculated. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell. The test article was considered to induce a positive response when the percentage of cells with aberrations are increased in a dose-response manner with one or more concentrations being statistically elevated relative to the solvent control group ($p \leq 0.05$).

Results

Statistical Results	"In both the presence and absence of metobolic activation ODB-2 caused no statistically significant increase in the proportion of metaphase figures containing chromosomal aberrations at any dose level when compared with the solvent control."
Remarks	With metabolic activation: no test concentration caused significant chromosome aberrations Without metabolic activation: no test concentration caused significant chromosome aberrations

Conclusions

Remarks: "Both positive control compounds caused large, statistically highly significant increases in chromosomal damage, demonstrating the sensitivity of this test system and the efficacy of the S-9 mix.

It is concluded that ODB-2 has shown no evidence of clastogenic activity in this *in vitro* cytogenetic test system."

Data Quality

Remarks: None

References

Huntingdon Research Centre, Ltd., "Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured *In Vitro* and Treated with ODB-2," January 9, 1989.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 6'(diethylamino)-3'-methyl-2'-(2,4-dimethylphenylamino)-

Remarks: Black XV, (CAS No. 36431-22-8), Purity 98.6%

Method

Method	*Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (McCann *et al*., 1975; McCann and Ames, 1976)

Test Type	*Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay and *Escherichia Coli* WP2 *uvr*A Reverse Mutation Assay
System of Testing	*Salmonella typhimurium* and *Escherichia Coli* WP2 *uvr*A
GLP (Yes/No)	Yes
Year	1993
Species/Strain	*Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, TA1537, and TA 1538 *Escherichia Coli* tester strain WP2 *uvr*A
Metobolic Activation	Species and cell type: Aroclor 1254 – induced rat liver S-9 (male Sprague-Dawley rat) Quantity: 500 µl to 2 ml of molten selective top agar Induced or not induced: Studied induced and non-induced cells
Concentrations Tested	100, 333, 1000, 3333, and 5000 µg/per plate (Plating aliquot 50 µl)
Statistical Methods	"For all replicate platings, the mean revertants per plate and the standard deviation will be calculated."
Remarks	● Number of Replicates: triplicate ● Positive Controls: TA98, TA100, TA1535, TA1537, TA1538, WP2 *urv*A: 2-aminoanthracene (with S-9 activation), TA98, TA1538: 2-nitrofluorene (without S-9 activation), TA100, TA1535: sodium azide (without S-9 activation), TA1537: 9-aminoacridine (without S-9 activation), WP2 *urv*A: methyl methanesulfonate (without S-9 activation) ● Criteria for Judging the Results: "For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article. Data sets for strains TA1535, TA1537 and TA1538 will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than three times the mean vehicle control value. Data sets for strains TA98, TA100 and WP2 *urv*A will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than two times the mean vehicle control value."

Results

Genotoxic effects concentration	With metabolic activation: no test concentration caused a positive response in the mutagenicicty assay Without metabolic activation: no test concentration caused a positive response in the mutagenicicty assay
Statistical Results	No appreciable toxicity was observed. No positive responses were observed.
Remarks	The test article solvent vehicle was dimethyl sulfoxide (DMSO).

Conclusions

Remarks: "The results of the *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) and *Escherichia coli* WP2 *uvr*A Reverse Mutation Assay with a Confirmatory Assay indicate that under the conditions of this study, Black XV did not cause a positive response with any of the tester strains in the presence and absence of Aroclor-induced rat liver S9."

Data Quality

Remarks: None

References

Microbiological Associates, Inc., "*Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) and *Escherichia coli* WP2 *uvr*A Reverse Mutation Assay with a Confirmatory Assay," August 27, 1993.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(diethylamino)-3'-methyl-2'-(phenylamino)-

Remarks: N-102, (CAS No. 29512-49-0), Purity 99%

Method

Method	*Salmonella*/*Escherichia Coli* Preincubation Mutagenicity Assay with Confirmatory Assay (McCann *et al*., 1975; McCann and Ames, 1976)
Test Type	*Salmonella*/*Escherichia Coli* Preincubation Mutagenicity Assay with Confirmatory Assay

System of Testing	*Salmonella typhimurium* and *Escherichia Coli* WP2 *uvr*A
GLP (Yes/No)	Yes
Year	1995
Species/Strain	*Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, TA1537 *Escherichia coli* tester strain WP2 *uvr*A (pKM101) and WP2 (pKM101)
Metobolic Activation	Species and cell type: Aroclor 1254 – induced rat liver S-9 (male Sprague-Dawley rat) Quantity: 0.5 ml Induced or not induced: Studied induced and non-induced cells
Concentrations Tested	10, 33, 100, 333, and 1000 µg/per plate (Plating aliquot 50 µl)
Statistical Methods	"For all replicate platings, the mean revertants per plate and the standard deviation will be calculated."
Remarks	● Number of Replicates: triplicate ● Positive Controls: All *Salmonella* Strains, WP2 *uvr* A (pKM101): 2-aminoanthrane (with S-9 activation), WP2 (pKM101): sterigmatocystin (with S-9 activation), TA98: 2-nitrofluorene (without S-9 activation), TA100, TA1535: sodium azide (without S-9 activation), TA1537: 9-aminoacridine (without S-9 activation), Both *E. coli* strains: methyl methansulfonate (without S-9 activation) ● Criteria for Judging the Results: "For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article. Data sets for strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than three times the mean vehicle control value. Data sets for strains TA98, TA100 and WP2 *urv*A (pKM101), and WP2 (pKM101) were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than two times the mean vehicle control value."

Results

Genotixic effects concentration	With metabolic activation: no test concentration caused a positive response in the mutagenicicty assay Without metabolic activation: no test concentration caused a positive response in the mutagenicity assay

Statistical Results	No appreciable toxicity was observed. No positive responses were observed.
Remarks	The test article solvent vehicle was dimethyl sulfoxide (DMSO).

Conclusions

Remarks: "All criteria for a valid study were met as described in the protocol. The results of the *Salmonella*/*Escherichia coli* Mutagenicity indicate that under the conditions of this study, N-102 did not cause a positive response with any of the tester strains in the presence and absence of Aroclor-induced rat liver S9."

Data Quality

Remarks: None

References

Microbiological Associates, Inc., "*Salmonella*/*Escherichia coli* Preincubation Mutagenicity Assay with a Confirmatory Assay," November 30,1995.

Other

None

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99%

Method

Method	*Salmonella*/*Escherichia Coli* Preincubation Mutagenicity Assay with Confirmatory Assay (McCann *et al*., 1975; McCann and Ames, 1976)
Test Type	*Salmonella*/*Escherichia Coli* Preincubation Mutagenicity Assay with Confirmatory Assay
System of Testing	*Salmonella typhimurium* and *Escherichia Coli* WP2 *uvr*A
GLP (Yes/No)	Yes
Year	1995

Species/Strain	*Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, TA1537 *Escherichia coli* tester strain WP2 *uvr*A (pKM101) and WP2 (pKM101)
Metobolic Activation	Species and cell type: Aroclor 1254 – induced rat liver S-9 (male Sprague-Dawley rat) Quantity: 0.5 ml Induced or not induced: Studied induced and non-induced cells
Concentrations Tested	10, 33, 100, 333, and 1000 µg/per plate (Plating aliquot 50 µl)
Statistical Methods	"For all replicate platings, the mean revertants per plate and the standard deviation will be calculated."
Remarks	● Number of Replicates: triplicate ● Positive Controls: All *Salmonella* Strains, WP2 *uvr* A (pKM101): 2-aminoanthrane (with S-9 activation), WP2 (pKM101): sterigmatocystin (with S-9 activation), TA98: 2-nitrofluorene (without S-9 activation), TA100, TA1535: sodium azide (without S-9 activation), TA1537: 9-aminoacridine (without S-9 activation), Both *E. coli* strains: methyl methansulfonate (without S-9 activation) ● Criteria for Judging the Results: "For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article. Data sets for strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than three times the mean vehicle control value. Data sets for strains TA98, TA100 and WP2 *urv*A (pKM101), and WP2 (pKM101) were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than two times the mean vehicle control value."

Results

Genotixic effects concentration	With metabolic activation: no test concentration caused a positive response in the mutagenicicty assay Without metabolic activation: no test concentration caused a positive response in the mutagenicity assay
Statistical Results	No appreciable toxicity was observed. No positive responses were observed.
Remarks	The test article solvent vehicle was dimethyl sulfoxide (DMSO).

Conclusions

Remarks: “All criteria for a valid study were met as described in the protocol. The results of the *Salmonella*/*Escherichia coli* Preincubation Mutagenicity Assay with Confirmatory Assay indicate that under the conditions of this study, ODB-2 did not cause a positive response with any of the tester strains in the presence and absence of Aroclor-induced rat liver S9.”

Data Quality

Remarks: None

References

Microbiological Associates, Inc., “*Salmonella*/*Escherichia coli* Preincubation Mutagenicity Assay with a Confirmatory Assay,” December 14,1995.

Other

None

21. Repeated Dose Toxicity

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity: not provided in the study - the estimated purity based on process knowledge is >98%.

Method

Method	OECD Guidelines for Testing Chemicals No. 412
Test Type	28 Day Oral Toxicity Study in Rats
GLP (Yes/No)	Yes
Year	1989
Species/Strain	Sprague-Dawley (CD) Rats, CD (SD) BR strain
Route of Administration	The test substance was administered by oral gavage to rats using a syringe and rubber catheter.
Duration of test	28 days
Doses/concentration levels	Dose volume: 10 ml/kg/day Dose Levels: 62.5, 250, and 1000 mg/kg/day
Sex	24 male and 24 female

Exposure period	"Animals were treated once daily, seven days per week for four weeks."
Frequency of treatment	"Animals were treated once daily, seven days per week for four weeks. Each animal received a constant dosage level based on its most recent recorded bodyweight."
Control group and treatment	Control animals similarly received 1% methylcellulose (MC) (10 ml/kg/day), 5 male and 5 female rats
Post exposure observation period	"All animals were observed daily for signs of ill health, behavioral changes or toxicosis. Any observed changes were recorded. All animals were checked early in each working day and again in the late afternoon to look for dead or moribund animals. This allowed a post mortem examination to be undertaken during the working part of that day. At weekends a similar procedure was followed except that the final check was carried out at mid-day."
Statistical methods	"The following sequence of statistical tests was used for bodyweight, organ weight and clinical pathology data: If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of values different from the mode was analyzed by appropriate methods. Otherwise: Bartlestt's test was applied to test for heterogeneity of variance between treatments. Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained. If no significant heterogeneity was detected (Or if a satisfactory transformation was found), a one-way analysis was present, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks was used. Analyses of variance were followed by Student's 't' test and Wiliams' test for a dose related response, although only the one thought more appropriate for the response pattern observed was reported. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of the 't' test and Williams' test (Shirley's test). For organ weight data, where appropriate, analysis of covariance was used in place of analysis of variance in the above sequence. The final body weight was used as covariate in an attempt to allow for differences in bodyweight which might have influenced the organ weights."
Remarks	● Age: 28 days old, rats used weighed 65 - 79 grams

	● No. of animals per sex per dose: 24 males, 24 females ● Vehicle: methylcellulose ● Clinical observations performed and frequency: All animals were observed daily for signs of ill health, behavioral changes or toxicosis above ● Organs examined at necropsy: adrenals, heart, kidneys, liver, spleen, any other macroscopically abnormal tissue

Results

NOAEL (NOEL)	"The dosage level of ODB-2 at which no signs of toxicity were recorded is therefore considered to be 1000 mg/kg/day."
LOAEL (LOEL)	"No changes were noted in the parameters and tissues examined that were considered to be related to treatment with ODB-2."
Actual dose received by dose level by sex	62.5, 250, and 1000 mg/kg/day
Toxic response/effects by dose level	No toxic response observed
Statistical results	No toxic responses
Remarks	● Body weight: Similar to those of control animals ● Food/water consumption: Similar to those of control animals ● Description, severity, time of onset and duration of clinical signs: No toxic responses ● Opthalmologic findings incidence and severity: Similar to those of control animals ● Hematological findings incidence and severity: Similar to those of control animals ● Clinical biochemistry findings incidence and severity: Similar to those of control animals ● Mortality and time to death: No toxic responses. One female rat died on day 14, but it was considered that the death of this animal occurred as a result of a dosing intubation error. ● Gross pathology incidence and severity: macroscopic abnormalities recorded for rats killed at termination were considered incidental and unrelated to treatment with ODB-2.

	● Organ weight changes: Significant lower adrenal weights were recorded for female rats receiving ODB-2, 1000 mg/kg/day in comparison with controls. This apparent shift to lower adrenal weights was not recorded for treated male rats and individual adrenal weights for treated female rats were generally within the expected weight range for this organ (adrenal weights, female rat: 5 percentile 44 mg, median 61 mg, 95 percentile 79 mg). In the absence of any other treatment related finding, the lower adrenal weights recorded for female rats in the high dosage group were therefore considered likely to have arisen by chance. ● Histopathology incidence and severity: No findings of toxicological significance.

Conclusions

Remarks: “The dosage level of ODB-2 at which no signs of toxicity were recorded is therefore considered to be 1000 mg/kg/day. No changes were noted in the parameters and tissues examined that were considered to be related to treatment with ODB-2.”

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., “Twenty-eight Day Oral Toxicity Study in Rats with ODB-2,” June 27, 1989.

Other

None

22. Toxicity to Reproduction

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	OECD Guidelines for Testing Chemicals No. 415
Test Type	One generation reproductive test
GLP (Yes/No)	Yes
Year	1993
Species/Strain	CD (SD) BR VAF/Plus strain
Route of Administration	Intragastric intubation
Doses/concentration levels	Dose volume: 1 ml/100 gram bodyweight (Suspension in 1% methylcellulose) Dose Levels: 0 (Control), 62.5, 250, and 1000 mg/kg/day
Sex	105 male and 105 female
Control group and treatment	24 male and 24 female Dose: 1 ml/100 gram bodyweight (1% methylcellulose)
Frequency of treatment	The once daily doses were administered ten weeks for males and two weeks for females prior to pairing, through pairing, pregnancy, and lactation up to sacrifice after weaning of their offspring. Apart from *in utero* exposure and possible contact through the mother's milk, offspring received no direct treatment with ODB-2.
Duration of test	21 weeks (21 day post partum)
Premating exposure period for males	10 weeks
Premating exposure period for females	2 weeks
Statistical methods	"All statistical analyses were carried out separately for males and females. Significance tests, employing analysis of variance following by an intergroup comparison with control, were performed on the following parameters and results are presented in relevant tables of the report: weekly bodyweight and female bodyweight change during pregnancy and lactation, food and water consumption, litter data and organ weights. Dependent on the heterogeneity of variance between treatment groups, parametric tests (analysis of variance (Snedecor and Cochran, 1967) followed by William's' test (Williams, 1971/2)) or non-parametric tests (Krkal-Wallis (Hollander and Wolfe, 1968) followed by Shirley's test (Shirley, 1977)) were used to analyze these data, as appropriate. For bodyweight and bodyweight change, the analyses were carried out using the individual animal as the experimental unit. Data relating to food and water consumption were analyses on a cage basis. For litter

	data, the basic sample unit was the litter and, due to the preponderance of non-normal distributions, non-parametric analyses were routinely used. Organ weight data were analyzed using body weight at post mortem as covariate, to allow for differences in bodyweight which may have influenced organ weight values. Where 75% or more of the values for a given variable were the same, a Fisher's exact test (Fisher, 1950) was used, when considered necessary. All significant (i.e. $P < 0.05$) intergroup differences from the control are reported and were supported by a significant analysis of variance ($P < 0.05$)."
Remarks	● Age: males, 4 weeks old, females, 7-8 weeks old, rats used weighed 72 - 95 grams ● No. of animals per sex per dose: 24 males, 24 females ● Vehicle: methylcellulose ● Dosing schedule and pre and post dosing observation periods: The once daily doses were administered ten weeks for males and two weeks for females prior to pairing, through pairing, pregnancy, and lactation up to sacrifice after weaning of their offspring. Observed through 21 day post partum. (21 weeks) ● Mating procedures (M/F ratios per cage, length of cohabitation, proof of pregnancy): One male and one female, 20 day mating period, daily vaginal smears to determine proof of pregnancy ● Standardization of litters: "For all litters, as soon as possible after parturition, the young were counted, individually identified within the litter by toe amputation, sexed, weighed and examined for external abnormalities. Keeping nest disturbance to a minimum litters were examined daily for dead and/or abnormal young. The pups were also weighed on Days 4, 8, 12, 16, and 21 post partum."

	● Clinical observations performed and frequency: Signs, Mortality, Bodyweight (weekly, during pregnancy Days 0, 7, 14, 17, and 20. Litter, Days 0, 7, 14, and 21 post partum), Food consumption (weekly), Water consumption (daily), Opthalmoscopy (prior to treatment), Pregnancy rate, Mating performance (7 days prior to mating and daily during 20 day mating period), Duration of pregnancy, Litter data, Offspring surface righting reflex, Offspring startle reflex, Offspring air righting reflex, Offspring pupil reflex, Terminal studies (adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostrate, testes, thymus).

Results

See the following results included below that were taken from the study identified above called: “ODB-2: A Study in the Rat on Reproductive Function of One Generation by Oral Administation.”

RESULTS

ANALYTICAL CHEMISTRY (See Analytical Chemistry Report)

The mean analysed concentrations of ODB-2 in dose formulations prepared for Day 1 of dosing at nominal concentrations of 2.5 and 10% w/v were close to nominal. At 0.625% w/v, the mean value was higher than expected (+18.6%) and therefore a further sample of this low dose level was taken on Day 2 of the study. The mean result on Day 2 and results in Weeks 11 and 16 were close to nominal.

ADULT ANIMALS

Clinical signs (Appendix 1)

There were no treatment-related clinical signs observed during the study.

Mortality (Table 1, Appendix 1)

One female receiving 1000 mg/kg/day (no. 186) was found dead during Week 11 (Day 3 of pairing) having shown no previous clinical signs; post mortem examination revealed moist red staining of the perioral and perinasal fur and severely congested, uncollapsed lungs but no apparent signs of intubation error. Male no. 93, also receiving 1000 mg/kg/day, was sacrificed Week 13 (Day 19 of pairing) after having previously been observed continually leaning to one side for nine days. Prior to sacrifice this animal appeared uncoordinated and continually rolled over; however, no abnormalities were detected at macroscopic post mortem examination.

One female in the low dose group (no. 134) was found dead Week 14 (Day 1 post partum) with red salivation and vaginal discharge. No previous clinical signs were recorded. Post mortem findings included enlarged cervical lymph nodes, congested thymus and lungs, gaseous distension of, and blood in, the stomach, dark contents in the small intestine. The uterus contained recently dead foetuses and placentae; thirteen pups had been born, all were cold and unfed when the dam was found.

As there were no trends or consistent pathology findings among these animals, the mortalities were considered to be coincidental and not related to treatment.

A further three female rats (no. 113 from control and nos. 169 and 191, 1000 mg/kg/day) were either found dead or were killed on the second day of treatment following loss of bodytone and laboured respiration. Post mortem examination revealed all three to have a perforated oesophagus. The clinical signs and necropsy findings were consistent with accidental intubation errors occurring during the dosing procedure. As one of the animals (no. 169) receiving 1000 mg/kg/day was found dead within 24 hours of the first dose, a replacement animal (no. 193) was selected for the duration of the study and dosed from this day. Data relating to no. 169 are not reported.

Bodyweight (Figures 1 and 2, Tables 2 and 3, Appendices 2 and 3)

Weekly bodyweight gain for males and females was generally similar for all groups ($P > 0.05$).

Bodyweight change for females during pregnancy and lactation was unaffected by treatment ($P > 0.05$).

Food consumption (Figure 3, Table 4, Appendix 4)

Food consumption for both sexes recorded during the pre-mating period (to Week 10) was essentially similar in all groups (cumulative intake $P > 0.05$).

Food conversion ratio (Table 5)

The efficiency of food utilisation during the pre-mating period, although slightly variable, was unaffected by treatment.

Water consumption (Figure 4, Table 6, Appendix 5)

Intergroup variation in water consumption during the periods recorded for males and females showed no dose relationship and cumulative values did not attain statistical significance ($P > 0.05$).

Ophthalmoscopy (Table 7, Appendix 6)

The type of ocular lesions seen were consistent with the age and strain of rat. There were no treatment-related changes.

Mating performance (Table 8, Appendix 7)

Mating performance, as assessed by the incidence and distribution of successful matings, the median pre-coital time and the type of smear recorded on the day of conception, was not adversely affected by treatment. The pregnancy rate was 100% for all groups with most females conceiving within the first four days after the start of pairing, corresponding with the expected length of an oestrous cycle.

The duration of pregnancy was similar for all groups ($P > 0.05$).

LITTER DATA

Litter values (Tables 9 and 10, Appendices 8 and 9)

Two females (one each at 62.5 and 250 mg/kg/day) resorbed their single implant. A single implantation is atypical and is generally considered insufficient to maintain pregnancy thus, in the absence of similar occurrences at the high dose level, the incidence was considered not to be treatment-related.

Among dams rearing young to weaning, mean values for implantation rates, pup survival, pup growth and associated sex ratio at birth and Day 21 post partum were similar for all groups ($P > 0.05$).

Pre-weaning development (Table 11, Appendix 10)

Mean ages of attainment of surface and air righting reflexes, startle response and presence of the pupil reflex at Day 20 post partum were similar for treated and control groups ($P > 0.05$).

TERMINAL STUDIES

Organ weights (Table 12, Appendix 11)

Intergroup differences in organ weights, adjusted for final bodyweight as appropriate, were only slight ($P > 0.05$) and revealed no clear or consistent changes in either sex which could be attributed to treatment.

Macroscopic pathology (Table 13, Appendix 1)

The macroscopic examination performed at terminal autopsy of surviving adults and offspring revealed no treatment-related changes.

Microscopic pathology (Table 14, Appendix 12)

No treatment-related findings were detected in the tissues examined.

No microscopic findings were detected which might have been associated with the failure of a small number of male/female pairings amongst rats receiving 62.5 or 250 mg/kg/day to produce offspring.

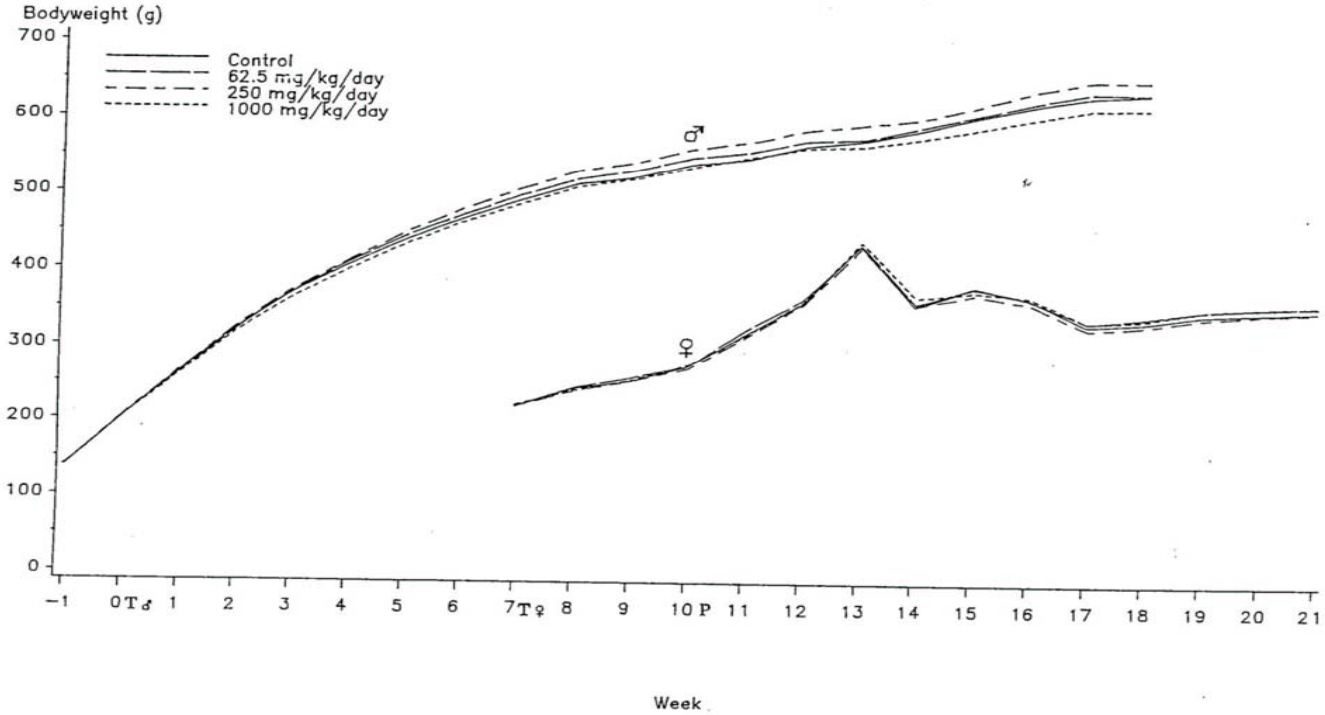
The few microscopic findings seen in the adult rats examined were considered to be spontaneous in origin and of no toxicological importance.

CONCLUSION

Based on the results obtained, this study indicated that dosages of 62.5, 250 or 1000 mg/kg/day were without adverse effect on the growth and reproductive capacity of male and female rats or the development of their offspring. The dosage of ODB-2 at which no signs of toxicity were recorded is therefore considered to be 1000 mg/kg/day.

FIGURE 1

Bodyweight - group mean values (g)



T Start of treatment
P Animals paired for 20 days

FIGURE 2

Bodyweight change of dams rearing young to weaning - group mean values (g)

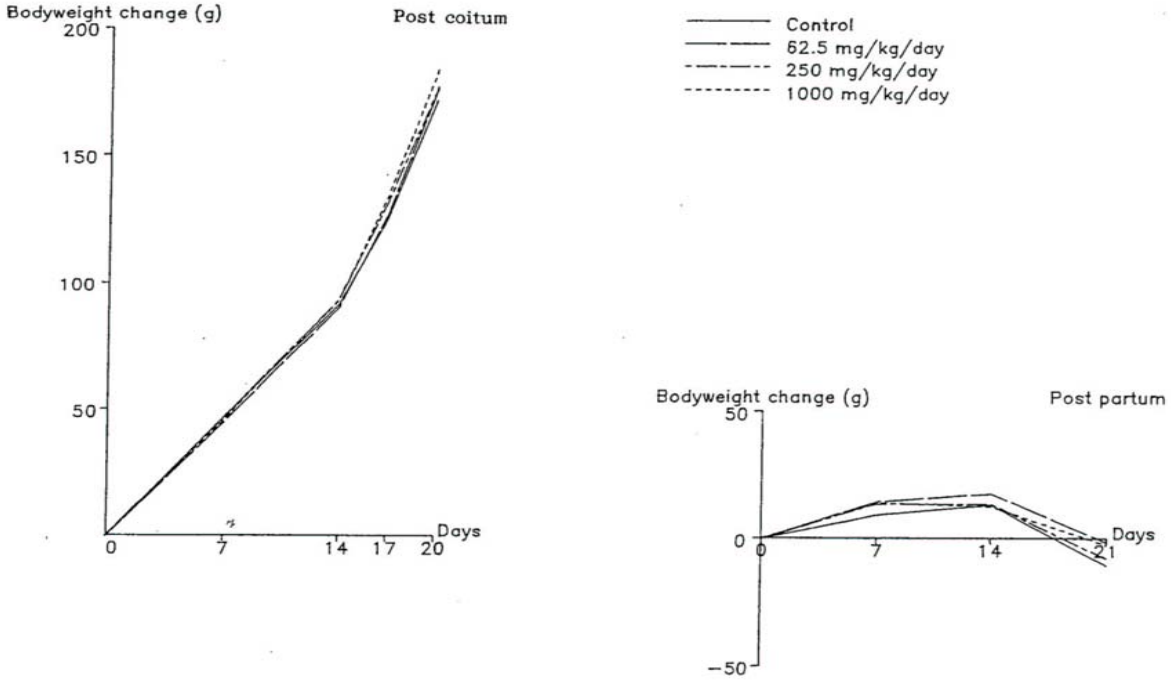


FIGURE 3

Food consumption - group mean values (g/rat/week)

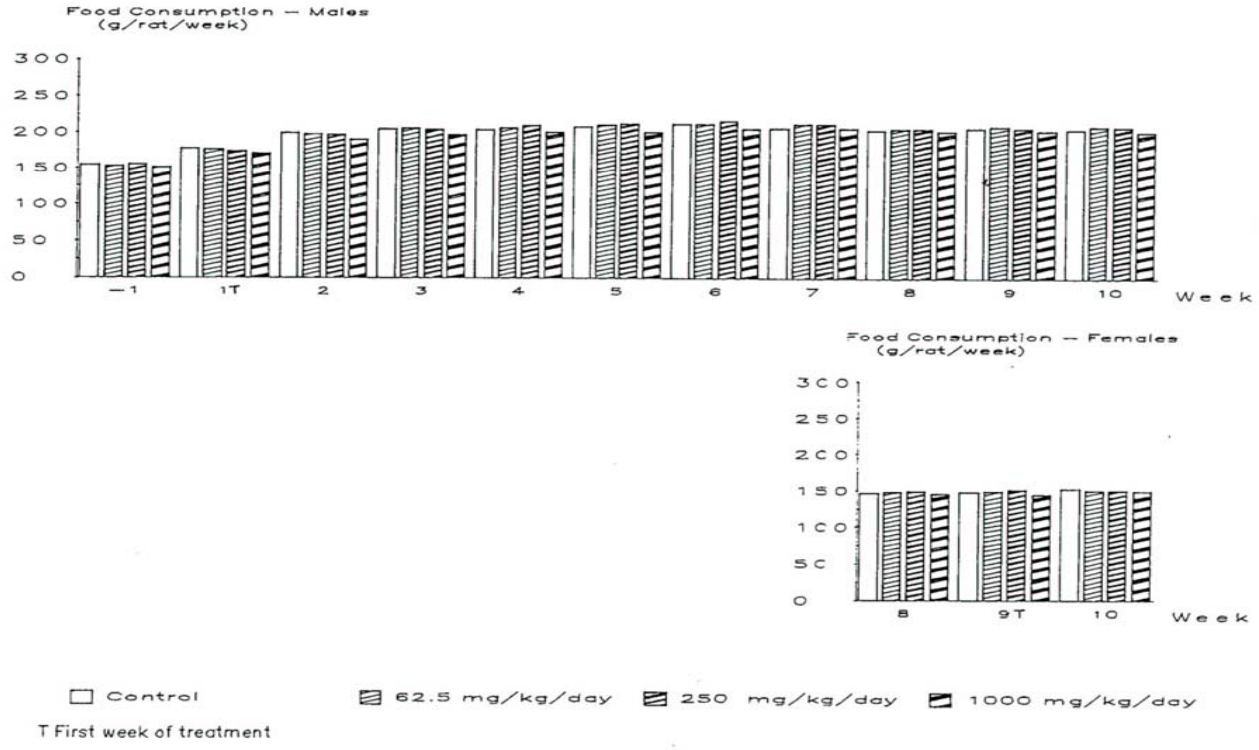


FIGURE 4

Water consumption - group mean values (g/rat/week)

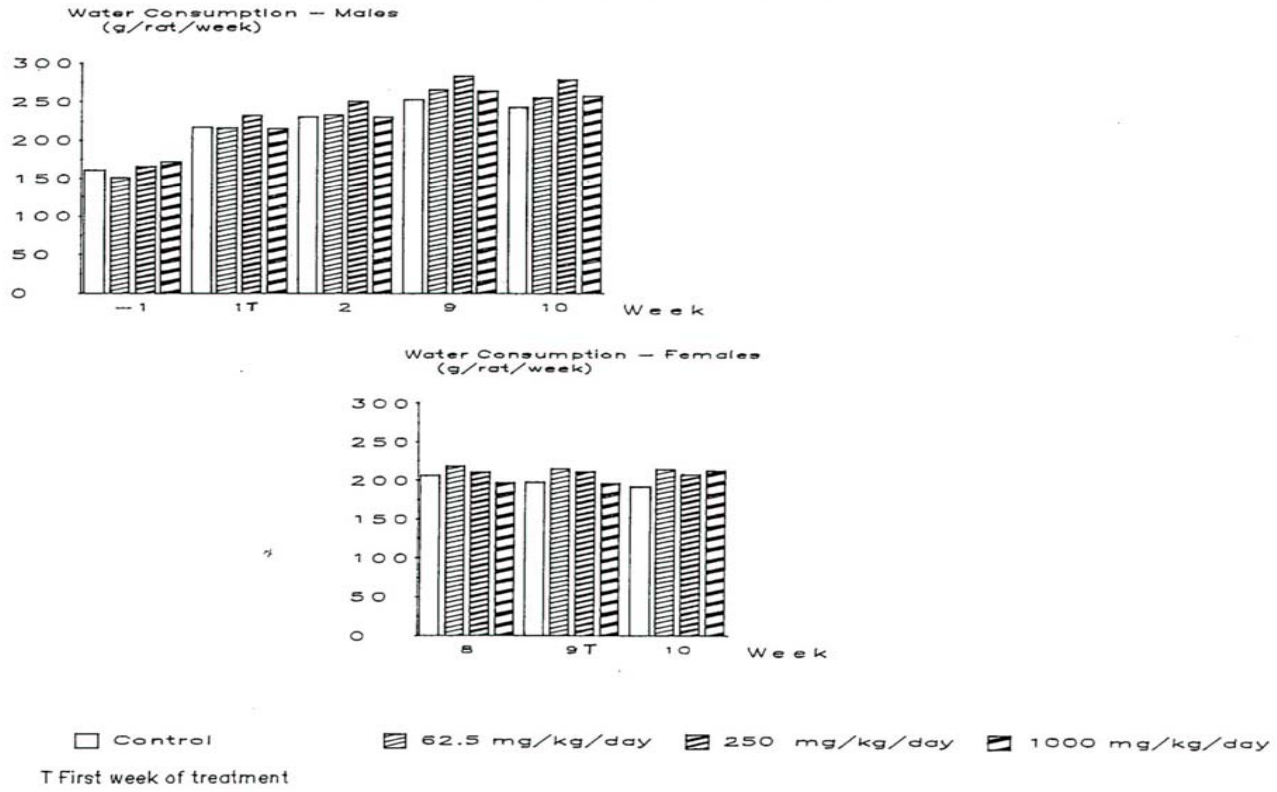


TABLE 1

Adult performance - summary of findings

Category	No. of animals in Group/dosage (mg/kg/day)			
	1 Control	2 62.5	3 250	4 1000
MALES				
Initial group size	24	24	24	24
Mortality: Day 19 of pairing	-	-	-	1b
Inducing pregnancy	23a	24	24	23ab
FEMALES				
Initial group size	24	24	24	24
Mortality: pre-pairing	1	-	-	1
Day 3 of pairing	-	-	-	1
Day 1 post partum	-	1	-	-
Total resorption	-	1	1	-
Rearing young to weaning	23	22	23	22

a One male not paired as female partner previously killed
b Male had induced pregnancy prior to sacrifice

TABLE 2

Bodyweight - group mean values (g)

Week	Group and dosage (mg/kg/day)								
	1♂ Control	2♂ 62.5	3♂ 250	4♂ 1000		1♀ Control	2♀ 62.5	3♀ 250	4♀ 1000
-1	137	138	139	138					
0T♂	203	202	204	203					
1	264	263	266	261					
2	322	321	324	318					
3	369	371	373	363					
4	406	410	412	400					
5	439	445	449	434					
6	467	472	480	462					
7	492	498	506	486		221	220	220	222
8T♀	514	520	531	510		244	246	242	243
9	523	532	542	521		255	259	255	256
10P	541	550	561	537		274	276	272	277
11	548	557	570	550		317	324	315	317
12	566	573	587	564		359	364	358	361
13	574	576	594	566		435	436	432	440
14	588	591	603	577		356	359	357	367
15	606	608	620	590		381	381	371	375
16	621	625	641	604		367	365	360	368
17	634	639	654	617		332	336	327	336
18						337	343	332	341
19						347	354	343	354
20						351	358	350	358
21						355	361	353	361
Bodyweight gain (g/rat):									
Weeks 0 - 17	431	437	451	415	Weeks 8 - 21	111	115	111	118
SD	78.5	52.4	77.9	63.4		15.1	19.8	14.9	19.2
% Control	-	101	105	96		-	104	100	106

No statistical significance ($P>0.05$)
SD Standard deviation
T Start of treatment
P Animals paired

TABLE 3

Bodyweight and bodyweight change of dams rearing young to weaning - group mean values (g)

Group/ dosage mg/kg/day	No. of animals	Bodyweight (g) at Day								
		post coitum					post partum			
		0	7	14	17	20	0	7	14	21
1 Control	23	281.7	328.2	373.0	406.1	453.6	360.9	370.1	374.2	350.5
2 62.5	22	289.9	334.5	380.1	416.4	466.5	359.0	373.5	376.5	357.6
3 250	23	279.2	325.8	372.7	410.8	456.3	355.3	368.9	368.9	347.3
4 1000	22	284.8	330.5	378.0	418.7	468.7	360.3	374.2	372.5	357.8

Group/ dosage mg/kg/day	No. of animals	Bodyweight change (g) relative to Day 0 (post coitum/post partum) at Day								
		post coitum					post partum			
		0	7	14	17	20	0	7	14	21
1 Control	23	0.0	46.5	91.3	124.4	171.8	0.0	9.3	13.3	-10.4
2 62.5	22	0.0	44.7	90.3	126.5	176.6	0.0	14.4	17.5	-1.5
3 250	23	0.0	46.6	93.5	131.6	177.2	0.0	13.6	13.6	-8.0
4 1000	22	0.0	45.8	93.2	134.0	183.9	0.0	14.0	12.3	-2.5

No statistical significance ($P>0.05$)

TABLE 4

Food consumption - group mean values (g/rat/week)

Week	Group and dosage (mg/kg/day)								
	1♂ Control	2♂ 62.5	3♂ 250	4♂ 1000		1♀ Control	2♀ 62.5	3♀ 250	4♀ 1000
-1	154	152	155	151					
1T♂	178	176	173	170					
2	199	197	196	190					
3	205	205	204	196					
4	204	207	209	200					
5	208	210	212	201					
6	212	212	215	205					
7	207	212	212	206					
8	204	206	206	202		146	148	148	145
9T♀	206	209	206	203		147	148	150	144
10	205	209	207	201		152	150	150	149
Cumulative intake (g/rat):									
Weeks 1 - 10	2026	2043	2042	1975	Weeks 9 - 10	299	298	301	294
SD	49.5	67.3	103.3	58.6		14.1	13.6	7.6	8.0
% Control	-	101	101	97		-	100	101	98

No statistical significance ($P>0.05$)
SD Standard deviation
T First week of treatment

TABLE 5

Food conversion ratio - group mean values

Week	Group and dosage (mg/kg/day)								
	1♂ Control	2♂ 62.5	3♂ 250	4♂ 1000		1♀ Control	2♀ 62.5	3♀ 250	4♀ 1000
1T♂	2.9	2.9	2.8	2.9					
2	3.4	3.4	3.4	3.3					
3	4.3	4.1	4.2	4.3					
4	5.6	5.2	5.4	5.4					
5	6.2	6.1	5.7	6.0					
6	7.8	7.7	7.1	7.1					
7	8.2	8.0	7.9	8.6					
8	9.3	9.3	8.3	8.5					
9T♀	22.1	18.3	18.4	18.7		13.0	11.6	11.3	10.7
10	11.4	11.7	11.1	12.7		7.7	8.9	8.7	7.1
Weeks 1 - 10	6.0	5.9	5.7	5.9	Weeks 9 - 10	9.7	10.0	9.9	8.5

Food conversion ratio = food consumption (g)/bwt gain (g)
T First week of treatment

TABLE 6

Water consumption - group mean values (g/rat/week)

Week	Group and dosage (mg/kg/day)							
	1♂ Control	2♂ 62.5	3♂ 250	4♂ 1000	1♀ Control	2♀ 62.5	3♀ 250	4♀ 1000
-1	184	177	189	183				
1T♂	206	199	218	209				
2	232	229	252	229				
8					200	218	215	205
9T♀	257	265	291	264	190	209	207	199
10	255	261	278	269	200	222	210	212
Cumulative intake (g/rat):								
Weeks 1 - 2	438	428	470	438				
SD	30.4	21.9	28.7	27.1				
% Control	-	98	107	100				
Weeks 9 - 10	512	526	568	533	391	431	416	413
SD	59.6	71.0	71.1	48.6	33.1	28.0	32.2	29.5
% Control	-	103	111	104	-	110	106	106

No statistical significance ($P>0.05$)
SD Standard deviation
T First week of treatment

TABLE 7

Ophthalmoscopy - summary of observations

Category	No. of animals in Group/dosage (mg/kg/day)			
	1 Control	2 62.5	3 250	4 1000
MALES				
Pre-treatment (Week -1)				
Examined	24	24	24	24
Vitreous: hyaloid remnant(s)	4	1	3	5
Pre-sacrifice (Week 17)				
Examined	24	24	24	23
Lens: posterior capsular opacity	-	-	1	-
Vitreous: hyaloid remnant	1	-	-	-
Retina: apparent hyperreflectivity	1	-	-	1
FEMALES				
Pre-treatment (Week 8)				
Examined	24	24	24	24
Vitreous: hyaloid remnant(s)	6	9	5	5
haemorrhage	-	1	-	-
Pre-sacrifice (Week 21)				
Examined	23	23	24	22
Vitreous: hyaloid remnant(s)	-	1	2	1
Retina: abnormality of optic nerve head	1	-	-	1

TABLE 8

Mating performance - group values

Group/ dosage (mg/kg/day)	No. of ♂/♀ pairs	Number conceiving on Day:									Preg rate %	Median pre-coital time (days)	Cell type at conception (from vaginal smear)					Duration of pregnancy (days)				
		1	2	3	4	6	7	15	U	Total			S	C	L (Day 1)	L	U	21	22	23	U	Mean
1 Control	23	12	3	2	6	-	-	-	-	23	100	1.0	22	-	1	-	-	3	14	5	1	22.1
2 62.5	24	9	2	8r	4	-	-	1	-	24	100	3.0	23r	1	-	-	-	4	16	3	-	22.0
3 250	24	5	10	8	-	-	-	-	1r	24	100	2.0	23	-	-	-	1r	4	17	2	-	21.9
4 1000	22d	7	7	3	2	2	1	-	-	22	100	2.0	18	2	-	2	-	2	16	2	2	22.0

Duration of pregnancy: no statistical significance ($P>0.05$)
d Excludes one dam found dead on Day 3 of pairing
r Includes one dam with total resorption
U Undetermined
Predominant cell type: S Sperm
C Cornified epithelial
L Leucocyte

TABLE 9

Litter data - group mean values

Group/ dosage mg/kg/day	No. of animals rearing young to weaning	Impl. sites	At birth						At Day 4				At Day 8			
			Implant loss %	Litter size		Pup loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)
				Total	Live											
1 Control	23	16.5	6.6	15.4	15.3	0.3	98.8	6.5	15.3	0.8	156.4	10.5	15.2	1.1	253.9	17.1
2 62.5	22	17.7	5.8	16.7	16.6	0.6	103.6	6.3	16.0	3.8	156.8	9.9	15.8	5.1	252.4	16.2
3 250	23	16.7	5.2	15.8	15.7	0.5	101.5	6.5	15.6	1.1	160.2	10.3	15.5	1.8	261.0	17.0
4 1000	22	17.2	4.3	16.5	16.4	0.8	106.6	6.5	16.0	2.5	162.4	10.2	15.8	4.0	262.3	16.7

Group/ dosage mg/kg/day	At Day 12				At Day 16				At Day 21			
	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)
1 Control	15.2	1.1	369.9	24.9	15.2	1.1	470.7	31.8	15.2	1.1	679.6	45.9
2 62.5	15.7	5.6	369.5	23.9	15.7	5.6	476.0	30.9	15.7	5.6	678.2	43.9
3 250	15.5	1.8	376.2	24.6	15.5	1.8	479.2	31.5	15.5	1.8	686.7	45.1
4 1000	15.7	4.4	374.5	24.0	15.7	4.4	480.4	30.8	15.7	4.4	703.0	45.1

No statistical significance ($P>0.05$)

TABLE 10

Sex ratio - group mean values

Group/ dosage mg/kg/day	No. of litters	At birth				At Day 21			
		Litter size				Litter size			
		♂	♀	Total	% Males	♂	♀	Total	% Males
1 Control	23	7.0	8.3	15.4	46.0	7.0	8.2	15.2	46.2
2 62.5	22	7.8	8.9	16.7	46.0	7.4	8.3	15.7	47.0
3 250	23	7.8	8.0	15.8	49.0	7.7	7.7	15.5	49.4
4 1000	22	7.8	8.7	16.5	46.9	7.5	8.2	15.7	47.1

No statistical significance for % males ($P>0.05$)

TABLE 11

Pre-weaning development - group mean values

Group/ dosage mg/kg/day	Duration of pregnancy (days)	Mean age (days post coitum) for attaining:			Pupil reflex (Day 20 post partum)
		Surface righting	Startle response	Air righting	% successful
1 Control	22.1	24.3	34.9	37.8	100
2 62.5	22.0	24.2	35.0	37.6	100
3 250	21.9	24.1	34.8	37.7	100
4 1000	22.0	24.3	34.6	37.7	100

No statistical significance ($P > 0.05$)

TABLE 12

Organ weights - group mean values

Group/ dosage mg/kg/day	No. of males	Body wt	Brain	Pitu-itary	Thymus	Heart	Lungs	Liver	Kidneys	Adrenals	Testes		Epididymides		Sem Ves/ prostate/ coag. gland
											Left	Right	Left	Right	
		g	g	mg	g	g	g	g	g	mg	g	g	g	g	g
Unadjusted values															
1 Control	24	628	2.1	15.4	0.29	1.91	2.01	26.9	4.83	57.5	1.772	1.785	0.685	0.712	3.199
2 62.5	24	632	2.2	15.6	0.31	1.91	2.06	27.2	4.75	59.6	1.827	1.826	0.685	0.713	3.432
3 250	24	646	2.1	15.4	0.30	1.90	2.06	25.9	4.70	60.1	1.847	1.831	0.677	0.713	3.232
4 1000	23	612	2.2	15.5	0.29	1.80	2.00	26.0	4.63	57.5	1.812	1.782	0.676	0.696	3.158
Adjusted values															
1			2.1	15.4	-	1.91	2.01	27.0	4.84	-	-	1.785	-	0.713	3.202
2			2.2	15.6	-	1.90	2.05	27.1	4.74	-	-	1.825	-	0.712	3.430
3			2.1	15.2	-	1.86	2.03	25.2	4.61	-	-	1.824	-	0.708	3.211
4			2.2	15.7	-	1.84	2.03	26.7	4.72	-	-	1.790	-	0.700	3.180

No statistical significance ($P>0.05$)

TABLE 12

(Organ weights - continued)

Group/ dosage mg/kg/day	No. of females	Body wt g	Brain g	Pitu-itary mg	Thymus g	Heart g	Lungs g	Liver g	Kidneys g	Adrenals mg	Ovaries mg
Unadjusted values											
1 Control	23	353	2.0	16.0	0.35	1.24	1.57	15.3	2.75	81.5	118.6
2 62.5	23	359	2.0	16.2	0.36	1.29	1.54	15.5	2.75	81.2	121.6
3 250	24	350	2.0	16.6	0.33	1.25	1.52	15.4	2.77	81.1	122.3
4 1000	22	358	2.0	15.8	0.33	1.27	1.60	16.1	2.81	85.3	127.4
Adjusted values											
1			2.0	-	0.35	1.25	1.57	15.4	2.76	-	-
2			2.0	-	0.36	1.29	1.53	15.3	2.73	-	-
3			2.0	-	0.33	1.26	1.53	15.6	2.79	-	-
4			2.0	-	0.33	1.26	1.59	15.9	2.79	-	-

No statistical significance ($P>0.05$)

TABLE 13

Macroscopic pathology - incidence summary

Removal Reason: Terminal	Group 1 Control	Group 2 62.5 mg/kg/day	Group 3 250 mg/kg/day	Group 4 1000 mg/kg/day
Males on study	24	24	24	24
Animals completed	24	24	24	23
Skin				
Scab/s	2	1	5	5
Skin				
Alopecia	2	2	5	5
Subcutis				
Clear fluid-filled cyst/s	0	0	0	1
Subcutaneous Mass				
Mass/es	1	0	0	0
Tail				
Swelling/s	0	0	1	0
Lymph Nodes - Cervical				
Enlarged	21	17	20	20
Congested	1	0	0	0
Thymus				
Small	0	0	0	1
Lungs				
Petechiae	3	2	5	2
Pale subpleural focus/i	1	0	1	4
Not collapsed	0	0	0	1
Congested	1	0	0	2
Dark subpleural foci	0	0	1	0
Heart				
Enlarged	1	0	0	0
Ventricles fenestrated	1	0	2	0
Pale area/s - atrium	1	0	0	0
White striae - ventricle/s	1	0	0	1
Adipose Tissue				
Torsioned nodule/s	0	2	0	1
Minimal	0	0	0	1
Congested	0	0	0	1
Yellow swelling/s - epididymal	0	1	0	0
Excessive	0	0	1	0
Liver				
Pale subcapsular area/s - median cleft	1	3	2	0
Enlarged	2	0	1	0
Lobe/s small	1	0	0	0
Spleen				
Adhesions	0	0	1	0
Capsule thickened area/s	1	0	0	0
Subcapsular mass	0	1	0	0

TABLE 13

(Macroscopic pathology - continued)

Removal Reason: Terminal	Group 1 Control	Group 2 62.5 mg/kg/day	Group 3 250 mg/kg/day	Group 4 1000 mg/kg/day
Males on study	24	24	24	24
Animals completed	24	24	24	23
Forestomach				
Limiting ridge thickened	1	0	0	0
Stomach Corpus Mucosa				
Gaseous cyst	1	0	0	0
Colon				
Contents soft	1	0	0	1
Kidneys				
Pale	1	0	0	0
Cortical depression/s	0	0	1	0
Enlarged	0	0	1	0
Seminal Vesicles				
Distended	0	1	2	0
Testes				
Small	0	2	1	1
Blue	0	2	1	1
Flaccid	0	2	1	0
Enlarged	0	3	1	0
Epididymides				
Small	0	2	1	1

TABLE 13

(Macroscopic pathology - continued)

Removal Reason: Terminal	Group 1 Control	Group 2 62.5 mg/kg/day	Group 3 250 mg/kg/day	Group 4 1000 mg/kg/day
Females on study	24	24	24	24
Animals completed	23	23	24	22
Skin				
Scab/s	0	0	2	3
Skin				
Alopecia	1	1	7	3
Pituitary				
A clear fluid-filled cyst	0	0	1	0
Raised focus	1	0	0	0
Dark focus	1	0	0	0
Incisors				
Pale	0	0	0	1
Lymph Nodes - Cervical				
Enlarged	18	17	17	17
Congested	0	0	2	0
Lungs				
Petechiae	1	2	4	0
Pale subpleural focus/i	7	1	1	0
Congested	2	1	4	3
Liver				
Pale subcapsular area/s - median cleft	3	4	2	2
Spleen				
Clear fluid-filled cyst/s	1	0	0	1
Adhesions	0	0	1	0
Capsule thickened area/s	1	0	0	0
Adrenals				
Enlarged	1	1	1	2
Dark subcapsular focus	1	0	0	0
Kidneys				
Uniform cortical scarring	0	1	0	0
Enlarged	0	1	0	0
Small	0	1	0	0
Yellow	0	1	0	0
Ovaries				
Clear fluid-filled cyst/s	1	0	0	0
Uterus				
Fluid distension	2	2	6	2
Skeletal Muscle				
Hernia	1	1	0	1

TABLE 14

Microscopic pathology - incidence summary

Males on study	Group 1 Control 24		Group 2 62.5 mg/kg/day 24		Group 3 250 mg/kg/day 24		Group 4 1000 mg/kg/day 24	
Animals completed	Decedent 0	Terminal 24	Decedent 0	Terminal 1	Decedent 0	Terminal 1	Decedent 1	Terminal 23
Prostate								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	14	0	1	0	1	0	17
Focal prostatitis (Total)	0	10	0	0	0	0	1	6
Minimal	0	10	0	0	0	0	1	6
Seminal Vesicles								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	24	0	1	0	1	1	23
Coagulating Gland								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	24	0	1	0	1	1	23
Epididymides								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	24	0	1	0	1	1	22
Unilateral absence of spermatozoa	0	0	0	0	0	0	0	1
Testes								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	24	0	1	0	1	1	22
Unilateral atrophy	0	0	0	0	0	0	0	1
Pituitary								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	24	0	1	0	1	1	22
Cyst(s) in pars anterior	0	0	0	0	0	0	0	1
Factors Contributory To Death								
Examined	0	0	0	0	0	0	1	0
Poor clinical condition	0	0	0	0	0	0	1	0

TABLE 14

(Microscopic pathology - continued)

Females on study	Group 1 Control 24		Group 2 62.5 mg/kg/day 24		Group 3 250 mg/kg/day 24		Group 4 1000 mg/kg/day 24	
Animals completed	Decedent 1	Terminal 23	Decedent 1	Terminal 1	Decedent 0	Terminal 1	Decedent 2	Terminal 22
Uterus								
Examined	1	23	1	1	0	1	2	22
No abnormalities detected	1	17	0	1	0	1	2	18
Luminal dilatation (Total)	0	6	0	0	0	0	0	4
Minimal	0	6	0	0	0	0	0	4
Placentae present	0	0	1	0	0	0	0	0
Cervix								
Examined	1	23	1	1	0	1	2	22
No abnormalities detected	1	23	1	1	0	1	2	22
Vagina								
Examined	1	23	1	1	0	1	2	22
No abnormalities detected	1	23	1	1	0	1	1	22
Inflammatory exudate in lumen (Total)	0	0	0	0	0	0	1	0
Moderate	0	0	0	0	0	0	1	0
Ovaries								
Examined	1	23	1	1	0	1	2	22
No abnormalities detected	1	22	1	1	0	1	2	22
Bilateral follicular cysts	0	1	0	0	0	0	0	0
Pituitary								
Examined	1	23	1	1	0	1	2	22
No abnormalities detected	1	21	1	1	0	1	2	22
Cyst(s) in pars anterior	0	2	0	0	0	0	0	0
Oesophagus								
Examined	0	1	0	0	0	0	0	0
No abnormalities detected	0	1	0	0	0	0	0	0
Factors Contributory To Death								
Examined	1	0	1	0	0	0	2	0
Unknown	0	0	1	0	0	0	1	0
Intubation error	1	0	0	0	0	0	1	0

Conclusions

Remarks: “Based on the result obtained, this study indicated that dosages of 62.5, 250, or 1000 mg/kg/day were without adverse effect on the growth and reproductive capacity of male and female rats or the development of their offspring. The dosage of ODB-2 at which no signs of toxicity were recorded is therefore considered to be 1000 mg/kg/day.”

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., “ODB-2: A Study in the Rat on Reproductive Function of One Generation by Oral Administation,” October 29, 1993.

Other

None

23. Developmental Toxicity

Test Substance

Identity: Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 6'-(dibutylamino)-3'-methyl-2'-(phenylamino)-

Remarks: ODB-2, (CAS No. 89331-94-2), Purity 99.6%

Method

Method	OECD Guidelines for Testing Chemicals No. 415
Test Type	One generation reproductive test
GLP (Yes/No)	Yes
Year	1993
Species/Strain	CD (SD) BR VAF/Plus strain
Route of Administration	Intragastric intubation
Doses/concentration levels	Dose volume: 1 ml/100 gram bodyweight (Suspension in 1% methylcellulose) Dose Levels: 0 (Control), 62.5, 250, and 1000 mg/kg/day
Sex	105 male and 105 female

Control group and treatment	24 male and 24 female Dose: 1 ml/100 gram bodyweight (1% methylcellulose)
Frequency of treatment	The once daily doses were administered ten weeks for males and two weeks for females prior to pairing, through pairing, pregnancy, and lactation up to sacrifice after weaning of their offspring. Apart from *in utero* exposure and possible contact through the mother's milk, offspring received no direct treatment with ODB-2.
Duration of test	21 weeks (21 day post partum)
Premating exposure period for males	10 weeks
Premating exposure period for females	2 weeks
Statistical methods	"All statistical analyses were carried out separately for males and females. Significance tests, employing analysis of variance following by an intergroup comparison with control, were performed on the following parameters and results are presented in relevant tables of the report: weekly bodyweight and female bodyweight change during pregnancy and lactation, food and water consumption, litter data and organ weights. Dependent on the heterogeneity of variance between treatment groups, parametric tests (analysis of variance (Snedecor and Cochran, 1967) followed by William's' test (Williams, 1971/2)) or non-parametric tests (Krkal-Wallis (Hollander and Wolfe, 1968) followed by Shirley's test (Shirley, 1977)) were used to analyze these data, as appropriate. For bodyweight and bodyweight change, the analyses were carried out using the individual animal as the experimental unit. Data relating to food and water consumption were analyses on a cage basis. For litter data, the basic sample unit was the litter and, due to the preponderance of non-normal distributions, non-parametric analyses were routinely used. Organ weight data were analyzed using body weight at post mortem as covariate, to allow for differences in bodyweight which may have influenced organ weight values. Where 75% or more of the values for a given variable were the same, a Fisher's exact test (Fisher, 1950) was used, when considered necessary. All significant (i.e. $P < 0.05$) intergroup differences from the control are reported and were supported by a significant analysis of variance ($P < 0.05$)."

Remarks	● Age: males, 4 weeks old, females, 7-8 weeks old, rats used weighed 72 - 95 grams ● No. of animals per sex per dose: 24 males, 24 females ● Vehicle: methylcellulose ● Dosing schedule and pre and post dosing observation periods: The once daily doses were administered ten weeks for males and two weeks for females prior to pairing, through pairing, pregnancy, and lactation up to sacrifice after weaning of their offspring. Observed through 21 day post partum. (21 weeks) ● Mating procedures (M/F ratios per cage, length of cohabitation, proof of pregnancy): One male and one female, 20 day mating period, daily vaginal smears to determine proof of pregnancy ● Standardization of litters: "For all litters, as soon as possible after parturition, the young were counted, individually identified within the litter by toe amputation, sexed, weighed and examined for external abnormalities. Keeping nest disturbance to a minimum litters were examined daily for dead and/or abnormal young. The pups were also weighed on Days 4, 8, 12, 16, and 21 post partum." ● Clinical observations performed and frequency: Signs, Mortality, Bodyweight (weekly, during pregnancy Days 0, 7, 14, 17, and 20. Litter, Days 0, 7, 14, and 21 post partum), Food consumption (weekly), Water consumption (daily), Opthalmoscopy (prior to treatment), Pregnancy rate, Mating performance (7 days prior to mating and daily during 20 day mating period), Duration of pregnancy, Litter data, Offspring surface righting reflex, Offspring startle reflex, Offspring air righting reflex, Offspring pupil reflex, Terminal studies (adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostrate, testes, thymus).

Results

See the following results included below that were taken from the study identified above called: "ODB-2: A Study in the Rat on Reproductive Function of One Generation by Oral Administation."

RESULTS

ANALYTICAL CHEMISTRY (See Analytical Chemistry Report)

The mean analysed concentrations of ODB-2 in dose formulations prepared for Day 1 of dosing at nominal concentrations of 2.5 and 10% w/v were close to nominal. At 0.625% w/v, the mean value was higher than expected (+18.6%) and therefore a further sample of this low dose level was taken on Day 2 of the study. The mean result on Day 2 and results in Weeks 11 and 16 were close to nominal.

ADULT ANIMALS

Clinical signs (Appendix 1)

There were no treatment-related clinical signs observed during the study.

Mortality (Table 1, Appendix 1)

One female receiving 1000 mg/kg/day (no. 186) was found dead during Week 11 (Day 3 of pairing) having shown no previous clinical signs; post mortem examination revealed moist red staining of the perioral and perinasal fur and severely congested, uncollapsed lungs but no apparent signs of intubation error. Male no. 93, also receiving 1000 mg/kg/day, was sacrificed Week 13 (Day 19 of pairing) after having previously been observed continually leaning to one side for nine days. Prior to sacrifice this animal appeared uncoordinated and continually rolled over; however, no abnormalities were detected at macroscopic post mortem examination.

One female in the low dose group (no. 134) was found dead Week 14 (Day 1 post partum) with red salivation and vaginal discharge. No previous clinical signs were recorded. Post mortem findings included enlarged cervical lymph nodes, congested thymus and lungs, gaseous distension of, and blood in, the stomach, dark contents in the small intestine. The uterus contained recently dead foetuses and placentae; thirteen pups had been born, all were cold and unfed when the dam was found.

As there were no trends or consistent pathology findings among these animals, the mortalities were considered to be coincidental and not related to treatment.

A further three female rats (no. 113 from control and nos. 169 and 191, 1000 mg/kg/day) were either found dead or were killed on the second day of treatment following loss of bodytone and laboured respiration. Post mortem examination revealed all three to have a perforated oesophagus. The clinical signs and necropsy findings were consistent with accidental intubation errors occurring during the dosing procedure. As one of the animals (no. 169) receiving 1000 mg/kg/day was found dead within 24 hours of the first dose, a replacement animal (no. 193) was selected for the duration of the study and dosed from this day. Data relating to no. 169 are not reported.

Bodyweight (Figures 1 and 2, Tables 2 and 3, Appendices 2 and 3)

Weekly bodyweight gain for males and females was generally similar for all groups ($P>0.05$).

Bodyweight change for females during pregnancy and lactation was unaffected by treatment ($P>0.05$).

Food consumption (Figure 3, Table 4, Appendix 4)

Food consumption for both sexes recorded during the pre-mating period (to Week 10) was essentially similar in all groups (cumulative intake $P>0.05$).

Food conversion ratio (Table 5)

The efficiency of food utilisation during the pre-mating period, although slightly variable, was unaffected by treatment.

Water consumption (Figure 4, Table 6, Appendix 5)

Intergroup variation in water consumption during the periods recorded for males and females showed no dose relationship and cumulative values did not attain statistical significance ($P>0.05$).

Ophthalmoscopy (Table 7, Appendix 6)

The type of ocular lesions seen were consistent with the age and strain of rat. There were no treatment-related changes.

Mating performance (Table 8, Appendix 7)

Mating performance, as assessed by the incidence and distribution of successful matings, the median pre-coital time and the type of smear recorded on the day of conception, was not adversely affected by treatment. The pregnancy rate was 100% for all groups with most females conceiving within the first four days after the start of pairing, corresponding with the expected length of an oestrous cycle.

The duration of pregnancy was similar for all groups ($P>0.05$).

LITTER DATA

Litter values (Tables 9 and 10, Appendices 8 and 9)

Two females (one each at 62.5 and 250 mg/kg/day) resorbed their single implant. A single implantation is atypical and is generally considered insufficient to maintain pregnancy thus, in the absence of similar occurrences at the high dose level, the incidence was considered not to be treatment-related.

Among dams rearing young to weaning, mean values for implantation rates, pup survival, pup growth and associated sex ratio at birth and Day 21 post partum were similar for all groups ($P>0.05$).

Pre-weaning development (Table 11, Appendix 10)

Mean ages of attainment of surface and air righting reflexes, startle response and presence of the pupil reflex at Day 20 post partum were similar for treated and control groups ($P > 0.05$).

TERMINAL STUDIES

Organ weights (Table 12, Appendix 11)

Intergroup differences in organ weights, adjusted for final bodyweight as appropriate, were only slight ($P > 0.05$) and revealed no clear or consistent changes in either sex which could be attributed to treatment.

Macroscopic pathology (Table 13, Appendix 1)

The macroscopic examination performed at terminal autopsy of surviving adults and offspring revealed no treatment-related changes.

Microscopic pathology (Table 14, Appendix 12)

No treatment-related findings were detected in the tissues examined.

No microscopic findings were detected which might have been associated with the failure of a small number of male/female pairings amongst rats receiving 62.5 or 250 mg/kg/day to produce offspring.

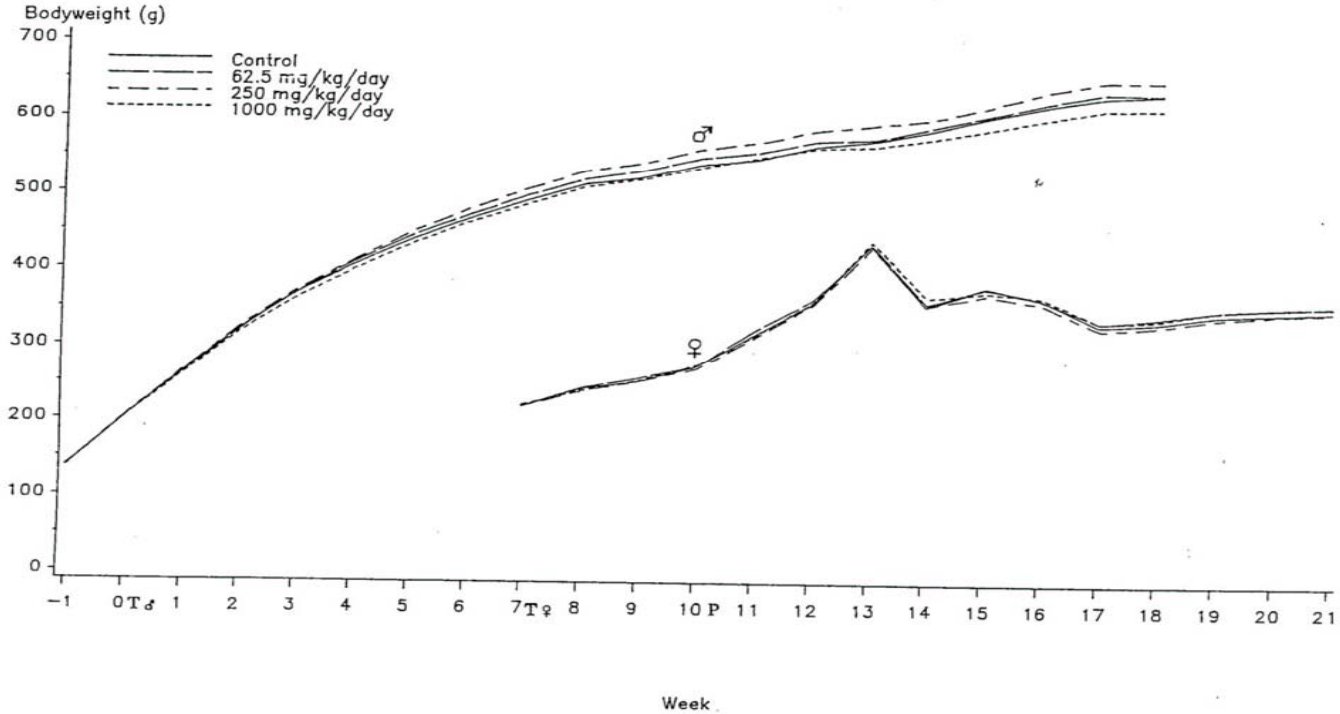
The few microscopic findings seen in the adult rats examined were considered to be spontaneous in origin and of no toxicological importance.

CONCLUSION

Based on the results obtained, this study indicated that dosages of 62.5, 250 or 1000 mg/kg/day were without adverse effect on the growth and reproductive capacity of male and female rats or the development of their offspring. The dosage of ODB-2 at which no signs of toxicity were recorded is therefore considered to be 1000 mg/kg/day.

FIGURE 1

Bodyweight - group mean values (g)



T Start of treatment
P Animals paired for 20 days

FIGURE 2

Bodyweight change of dams rearing young to weaning - group mean values (g)

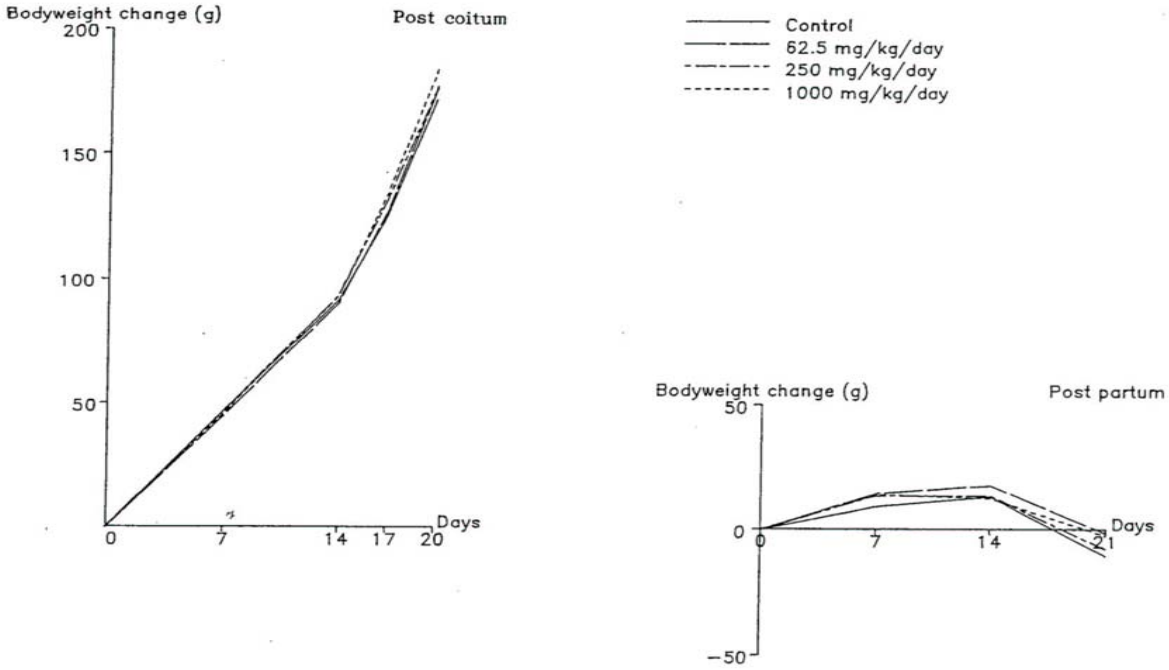
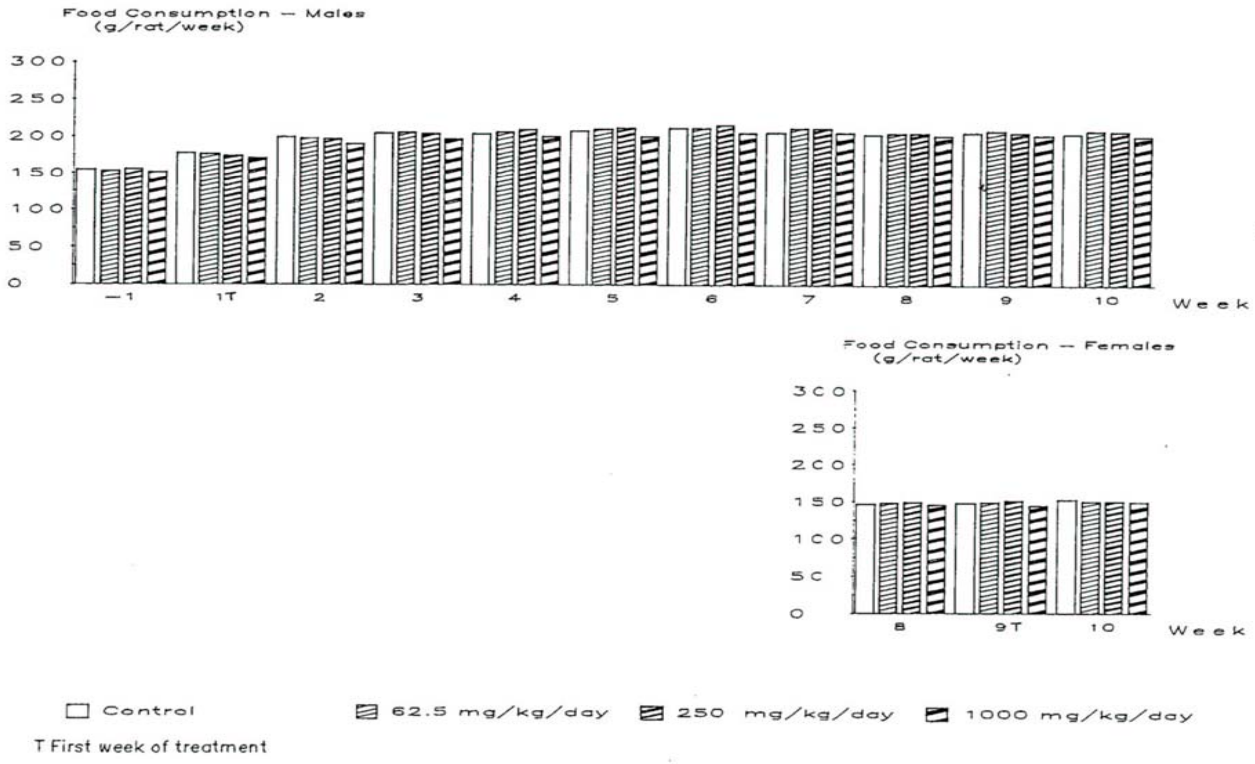


FIGURE 3

Food consumption - group mean values (g/rat/week)



T First week of treatment

FIGURE 4

Water consumption - group mean values (g/rat/week)

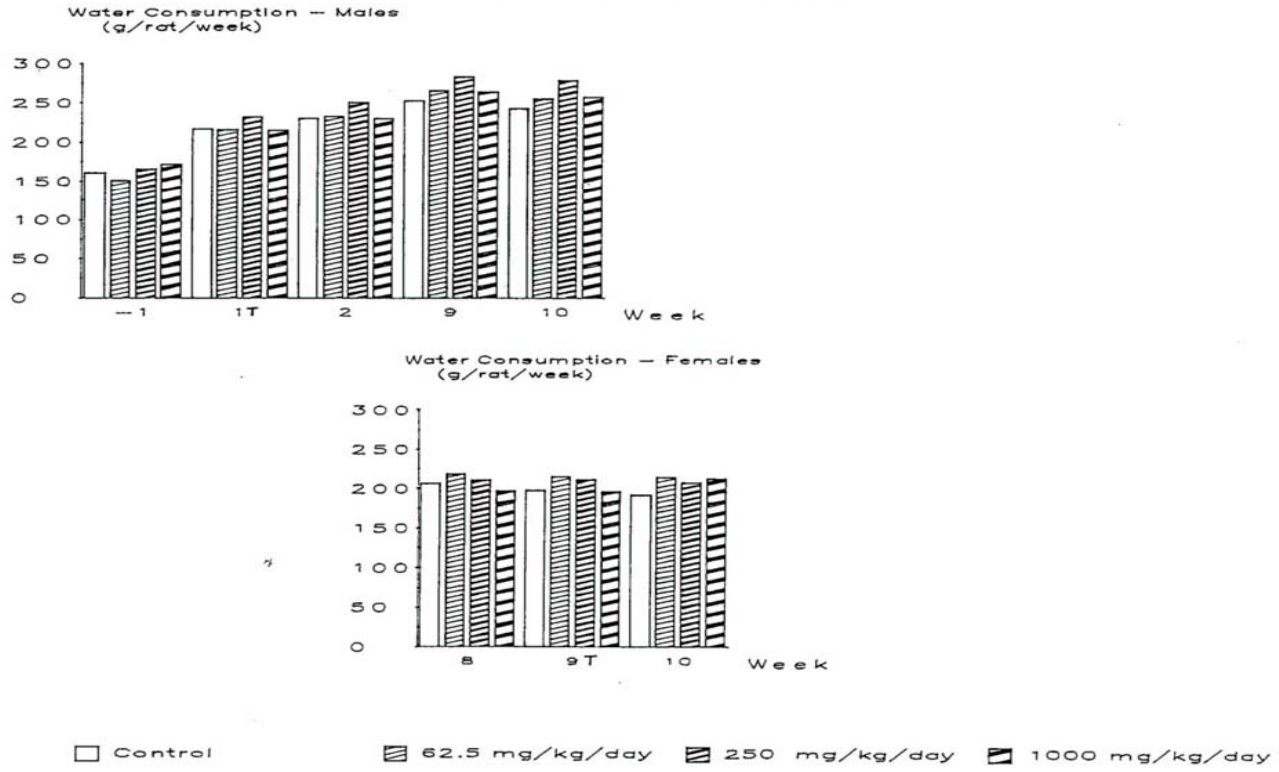


TABLE 1

Adult performance - summary of findings

Category	No. of animals in Group/dosage (mg/kg/day)			
	1 Control	2 62.5	3 250	4 1000
MALES				
Initial group size	24	24	24	24
Mortality: Day 19 of pairing	-	-	-	1b
Inducing pregnancy	23a	24	24	23ab
FEMALES				
Initial group size	24	24	24	24
Mortality: pre-pairing	1	-	-	1
Day 3 of pairing	-	-	-	1
Day 1 post partum	-	1	-	-
Total resorption	-	1	1	-
Rearing young to weaning	23	22	23	22

a One male not paired as female partner previously killed
b Male had induced pregnancy prior to sacrifice

TABLE 2

Bodyweight - group mean values (g)

Week	Group and dosage (mg/kg/day)								
	1♂ Control	2♂ 62.5	3♂ 250	4♂ 1000		1♀ Control	2♀ 62.5	3♀ 250	4♀ 1000
-1	137	138	139	138					
0T♂	203	202	204	203					
1	264	263	266	261					
2	322	321	324	318					
3	369	371	373	363					
4	406	410	412	400					
5	439	445	449	434					
6	467	472	480	462					
7	492	498	506	486		221	220	220	222
8T♀	514	520	531	510		244	246	242	243
9	523	532	542	521		255	259	255	256
10P	541	550	561	537		274	276	272	277
11	548	557	570	550		317	324	315	317
12	566	573	587	564		359	364	358	361
13	574	576	594	566		435	436	432	440
14	588	591	603	577		356	359	357	367
15	606	608	620	590		381	381	371	375
16	621	625	641	604		367	365	360	368
17	634	639	654	617		332	336	327	336
18						337	343	332	341
19						347	354	343	354
20						351	358	350	358
21						355	361	353	361
Bodyweight gain (g/rat):									
Weeks 0 - 17	431	437	451	415	Weeks 8 - 21	111	115	111	118
SD	78.5	52.4	77.9	63.4		15.1	19.8	14.9	19.2
% Control	-	101	105	96		-	104	100	106

No statistical significance ($P>0.05$)
SD Standard deviation
T Start of treatment
P Animals paired

TABLE 3

Bodyweight and bodyweight change of dams rearing young to weaning - group mean values (g)

Group/ dosage mg/kg/day	No. of animals	Bodyweight (g) at Day								
		post coitum					post partum			
		0	7	14	17	20	0	7	14	21
1 Control	23	281.7	328.2	373.0	406.1	453.6	360.9	370.1	374.2	350.5
2 62.5	22	289.9	334.5	380.1	416.4	466.5	359.0	373.5	376.5	357.6
3 250	23	279.2	325.8	372.7	410.8	456.3	355.3	368.9	368.9	347.3
4 1000	22	284.8	330.5	378.0	418.7	468.7	360.3	374.2	372.5	357.8

Group/ dosage mg/kg/day	No. of animals	Bodyweight change (g) relative to Day 0 (post coitum/post partum) at Day								
		post coitum					post partum			
		0	7	14	17	20	0	7	14	21
1 Control	23	0.0	46.5	91.3	124.4	171.8	0.0	9.3	13.3	-10.4
2 62.5	22	0.0	44.7	90.3	126.5	176.6	0.0	14.4	17.5	-1.5
3 250	23	0.0	46.6	93.5	131.6	177.2	0.0	13.6	13.6	-8.0
4 1000	22	0.0	45.8	93.2	134.0	183.9	0.0	14.0	12.3	-2.5

No statistical significance ($P>0.05$)

TABLE 4

Food consumption - group mean values (g/rat/week)

Week	Group and dosage (mg/kg/day)								
	1♂ Control	2♂ 62.5	3♂ 250	4♂ 1000		1♀ Control	2♀ 62.5	3♀ 250	4♀ 1000
-1	154	152	155	151					
1T♂	178	176	173	170					
2	199	197	196	190					
3	205	205	204	196					
4	204	207	209	200					
5	208	210	212	201					
6	212	212	215	205					
7	207	212	212	206					
8	204	206	206	202		146	148	148	145
9T♀	206	209	206	203		147	148	150	144
10	205	209	207	201		152	150	150	149
Cumulative intake (g/rat):									
Weeks 1 - 10	2026	2043	2042	1975	Weeks 9 - 10	299	298	301	294
SD	49.5	67.3	103.3	58.6		14.1	13.6	7.6	8.0
% Control	-	101	101	97		-	100	101	98

No statistical significance ($P>0.05$)
SD Standard deviation
T First week of treatment

TABLE 5

Food conversion ratio - group mean values

Week	Group and dosage (mg/kg/day)								
	1♂ Control	2♂ 62.5	3♂ 250	4♂ 1000		1♀ Control	2♀ 62.5	3♀ 250	4♀ 1000
1T♂	2.9	2.9	2.8	2.9					
2	3.4	3.4	3.4	3.3					
3	4.3	4.1	4.2	4.3					
4	5.6	5.2	5.4	5.4					
5	6.2	6.1	5.7	6.0					
6	7.8	7.7	7.1	7.1					
7	8.2	8.0	7.9	8.6					
8	9.3	9.3	8.3	8.5					
9T♀	22.1	18.3	18.4	18.7		13.0	11.6	11.3	10.7
10	11.4	11.7	11.1	12.7		7.7	8.9	8.7	7.1
Weeks 1 - 10	6.0	5.9	5.7	5.9	Weeks 9 - 10	9.7	10.0	9.9	8.5

Food conversion ratio = food consumption (g)/bwt gain (g)
T First week of treatment

TABLE 6

Water consumption - group mean values (g/rat/week)

Week	Group and dosage (mg/kg/day)							
	1♂ Control	2♂ 62.5	3♂ 250	4♂ 1000	1♀ Control	2♀ 62.5	3♀ 250	4♀ 1000
-1	184	177	189	183				
1T♂	206	199	218	209				
2	232	229	252	229				
8					200	218	215	205
9T♀	257	265	291	264	190	209	207	199
10	255	261	278	269	200	222	210	212
Cumulative intake (g/rat):								
Weeks 1 - 2	438	428	470	438				
SD	30.4	21.9	28.7	27.1				
% Control	-	98	107	100				
Weeks 9 - 10	512	526	568	533	391	431	416	413
SD	59.6	71.0	71.1	48.6	33.1	28.0	32.2	29.5
% Control	-	103	111	104	-	110	106	106

No statistical significance ($P>0.05$)
SD Standard deviation
T First week of treatment

TABLE 7

Ophthalmoscopy - summary of observations

Category	No. of animals in Group/dosage (mg/kg/day)			
	1 Control	2 62.5	3 250	4 1000
MALES				
Pre-treatment (Week -1)				
Examined	24	24	24	24
Vitreous: hyaloid remnant(s)	4	1	3	5
Pre-sacrifice (Week 17)				
Examined	24	24	24	23
Lens: posterior capsular opacity	-	-	1	-
Vitreous: hyaloid remnant	1	-	-	-
Retina: apparent hyperreflectivity	1	-	-	1
FEMALES				
Pre-treatment (Week 8)				
Examined	24	24	24	24
Vitreous: hyaloid remnant(s)	6	9	5	5
haemorrhage	-	1	-	-
Pre-sacrifice (Week 21)				
Examined	23	23	24	22
Vitreous: hyaloid remnant(s)	-	1	2	1
Retina: abnormality of optic nerve head	1	-	-	1

TABLE 8

Mating performance - group values

Group/ dosage (mg/kg/day)	No. of ♂/♀ pairs	Number conceiving on Day:									Preg rate %	Median pre-coital time (days)	Cell type at conception (from vaginal smear)					Duration of pregnancy (days)				
		1	2	3	4	6	7	15	U	Total			S	C	L (Day 1)	L	U	21	22	23	U	Mean
1 Control	23	12	3	2	6	-	-	-	-	23	100	1.0	22	-	1	-	-	3	14	5	1	22.1
2 62.5	24	9	2	8r	4	-	-	1	-	24	100	3.0	23r	1	-	-	-	4	16	3	-	22.0
3 250	24	5	10	8	-	-	-	-	1r	24	100	2.0	23	-	-	-	1r	4	17	2	-	21.9
4 1000	22d	7	7	3	2	2	1	-	-	22	100	2.0	18	2	-	2	-	2	16	2	2	22.0

Duration of pregnancy: no statistical significance ($P > 0.05$)

d Excludes one dam found dead on Day 3 of pairing

r Includes one dam with total resorption

U Undetermined

Predominant cell type: S Sperm
C Cornified epithelial
L Leucocyte

TABLE 9

Litter data - group mean values

Group/ dosage mg/kg/day	No. of animals rearing young to weaning	Impl. sites	At birth						At Day 4				At Day 8			
			Implant loss %	Litter size		Pup loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)
				Total	Live											
1 Control	23	16.5	6.6	15.4	15.3	0.3	98.8	6.5	15.3	0.8	156.4	10.5	15.2	1.1	253.9	17.1
2 62.5	22	17.7	5.8	16.7	16.6	0.6	103.6	6.3	16.0	3.8	156.8	9.9	15.8	5.1	252.4	16.2
3 250	23	16.7	5.2	15.8	15.7	0.5	101.5	6.5	15.6	1.1	160.2	10.3	15.5	1.8	261.0	17.0
4 1000	22	17.2	4.3	16.5	16.4	0.8	106.6	6.5	16.0	2.5	162.4	10.2	15.8	4.0	262.3	16.7

Group/ dosage mg/kg/day	At Day 12				At Day 16				At Day 21			
	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)
1 Control	15.2	1.1	369.9	24.9	15.2	1.1	470.7	31.8	15.2	1.1	679.6	45.9
2 62.5	15.7	5.6	369.5	23.9	15.7	5.6	476.0	30.9	15.7	5.6	678.2	43.9
3 250	15.5	1.8	376.2	24.6	15.5	1.8	479.2	31.5	15.5	1.8	686.7	45.1
4 1000	15.7	4.4	374.5	24.0	15.7	4.4	480.4	30.8	15.7	4.4	703.0	45.1

No statistical significance ($P > 0.05$)

TABLE 10

Sex ratio - group mean values

Group/ dosage mg/kg/day	No. of litters	At birth				At Day 21			
		Litter size				Litter size			
		♂	♀	Total	% Males	♂	♀	Total	% Males
1 Control	23	7.0	8.3	15.4	46.0	7.0	8.2	15.2	46.2
2 62.5	22	7.8	8.9	16.7	46.0	7.4	8.3	15.7	47.0
3 250	23	7.8	8.0	15.8	49.0	7.7	7.7	15.5	49.4
4 1000	22	7.8	8.7	16.5	46.9	7.5	8.2	15.7	47.1

No statistical significance for % males ($P>0.05$)

TABLE 11

Pre-weaning development - group mean values

Group/ dosage mg/kg/day	Duration of pregnancy (days)	Mean age (days post coitum) for attaining:			Pupil reflex (Day 20 post partum)
		Surface righting	Startle response	Air righting	% successful
1 Control	22.1	24.3	34.9	37.8	100
2 62.5	22.0	24.2	35.0	37.6	100
3 250	21.9	24.1	34.8	37.7	100
4 1000	22.0	24.3	34.6	37.7	100

No statistical significance (P>0.05)

TABLE 12

Organ weights - group mean values

Group/ dosage mg/kg/day	No. of males	Body wt	Brain	Pitu-itary	Thymus	Heart	Lungs	Liver	Kidneys	Adrenals	Testes		Epididymides		Sem Ves/ prostate/ coag. gland
											Left	Right	Left	Right	
		g	g	mg	g	g	g	g	g	mg	g	g	g	g	g
Unadjusted values															
1 Control	24	628	2.1	15.4	0.29	1.91	2.01	26.9	4.83	57.5	1.772	1.785	0.685	0.712	3.199
2 62.5	24	632	2.2	15.6	0.31	1.91	2.06	27.2	4.75	59.6	1.827	1.826	0.685	0.713	3.432
3 250	24	646	2.1	15.4	0.30	1.90	2.06	25.9	4.70	60.1	1.847	1.831	0.677	0.713	3.232
4 1000	23	612	2.2	15.5	0.29	1.80	2.00	26.0	4.63	57.5	1.812	1.782	0.676	0.696	3.158
Adjusted values															
1			2.1	15.4	-	1.91	2.01	27.0	4.84	-	-	1.785	-	0.713	3.202
2			2.2	15.6	-	1.90	2.05	27.1	4.74	-	-	1.825	-	0.712	3.430
3			2.1	15.2	-	1.86	2.03	25.2	4.61	-	-	1.824	-	0.708	3.211
4			2.2	15.7	-	1.84	2.03	26.7	4.72	-	-	1.790	-	0.700	3.180

No statistical significance (P>0.05)

TABLE 12

(Organ weights - continued)

Group/ dosage mg/kg/day	No. of females	Body wt g	Brain g	Pitu- itary mg	Thymus g	Heart g	Lungs g	Liver g	Kidneys g	Adrenals mg	Ovaries mg
Unadjusted values											
1 Control	23	353	2.0	16.0	0.35	1.24	1.57	15.3	2.75	81.5	118.6
2 62.5	23	359	2.0	16.2	0.36	1.29	1.54	15.5	2.75	81.2	121.6
3 250	24	350	2.0	16.6	0.33	1.25	1.52	15.4	2.77	81.1	122.3
4 1000	22	358	2.0	15.8	0.33	1.27	1.60	16.1	2.81	85.3	127.4
Adjusted values											
1			2.0	-	0.35	1.25	1.57	15.4	2.76	-	-
2			2.0	-	0.36	1.29	1.53	15.3	2.73	-	-
3			2.0	-	0.33	1.26	1.53	15.6	2.79	-	-
4			2.0	-	0.33	1.26	1.59	15.9	2.79	-	-

No statistical significance ($P>0.05$)

TABLE 13

Macroscopic pathology - incidence summary

Removal Reason: Terminal	Group 1 Control	Group 2 62.5 mg/kg/day	Group 3 250 mg/kg/day	Group 4 1000 mg/kg/day
Males on study	24	24	24	24
Animals completed	24	24	24	23
Skin				
Scab/s	2	1	5	5
Skin				
Alopecia	2	2	5	5
Subcutis				
Clear fluid-filled cyst/s	0	0	0	1
Subcutaneous Mass				
Mass/es	1	0	0	0
Tail				
Swelling/s	0	0	1	0
Lymph Nodes - Cervical				
Enlarged	21	17	20	20
Congested	1	0	0	0
Thymus				
Small	0	0	0	1
Lungs				
Petechiae	3	2	5	2
Pale subpleural focus/i	1	0	1	4
Not collapsed	0	0	0	1
Congested	1	0	0	2
Dark subpleural foci	0	0	1	0
Heart				
Enlarged	1	0	0	0
Ventricles fenestrated	1	0	2	0
Pale area/s - atrium	1	0	0	0
White striae - ventricle/s	1	0	0	1
Adipose Tissue				
Torsioned nodule/s	0	2	0	1
Minimal	0	0	0	1
Congested	0	0	0	1
Yellow swelling/s - epididymal	0	1	0	0
Excessive	0	0	1	0
Liver				
Pale subcapsular area/s - median cleft	1	3	2	0
Enlarged	2	0	1	0
Lobe/s small	1	0	0	0
Spleen				
Adhesions	0	0	1	0
Capsule thickened area/s	1	0	0	0
Subcapsular mass	0	1	0	0

TABLE 13

(Macroscopic pathology - continued)

Removal Reason: Terminal	Group 1 Control	Group 2 62.5 mg/kg/day	Group 3 250 mg/kg/day	Group 4 1000 mg/kg/day
Males on study	24	24	24	24
Animals completed	24	24	24	23
Forestomach				
Limiting ridge thickened	1	0	0	0
Stomach Corpus Mucosa				
Gaseous cyst	1	0	0	0
Colon				
Contents soft	1	0	0	1
Kidneys				
Pale	1	0	0	0
Cortical depression/s	0	0	1	0
Enlarged	0	0	1	0
Seminal Vesicles				
Distended	0	1	2	0
Testes				
Small	0	2	1	1
Blue	0	2	1	1
Flaccid	0	2	1	0
Enlarged	0	3	1	0
Epididymides				
Small	0	2	1	1

TABLE 13

(Macroscopic pathology - continued)

Removal Reason: Terminal	Group 1 Control	Group 2 62.5 mg/kg/day	Group 3 250 mg/kg/day	Group 4 1000 mg/kg/day
Females on study	24	24	24	24
Animals completed	23	23	24	22
Skin				
Scab/s	0	0	2	3
Skin				
Alopecia	1	1	7	3
Pituitary				
A clear fluid-filled cyst	0	0	1	0
Raised focus	1	0	0	0
Dark focus	1	0	0	0
Incisors				
Pale	0	0	0	1
Lymph Nodes - Cervical				
Enlarged	18	17	17	17
Congested	0	0	2	0
Lungs				
Petechiae	1	2	4	0
Pale subpleural focus/i	7	1	1	0
Congested	2	1	4	3
Liver				
Pale subcapsular area/s - median cleft	3	4	2	2
Spleen				
Clear fluid-filled cyst/s	1	0	0	1
Adhesions	0	0	1	0
Capsule thickened area/s	1	0	0	0
Adrenals				
Enlarged	1	1	1	2
Dark subcapsular focus	1	0	0	0
Kidneys				
Uniform cortical scarring	0	1	0	0
Enlarged	0	1	0	0
Small	0	1	0	0
Yellow	0	1	0	0
Ovaries				
Clear fluid-filled cyst/s	1	0	0	0
Uterus				
Fluid distension	2	2	6	2
Skeletal Muscle				
Hernia	1	1	0	1

TABLE 14

Microscopic pathology - incidence summary

Males on study	Group 1 Control 24		Group 2 62.5 mg/kg/day 24		Group 3 250 mg/kg/day 24		Group 4 1000 mg/kg/day 24	
Animals completed	Decedent 0	Terminal 24	Decedent 0	Terminal 1	Decedent 0	Terminal 1	Decedent 1	Terminal 23
Prostate								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	14	0	1	0	1	0	17
Focal prostatitis (Total)	0	10	0	0	0	0	1	6
Minimal	0	10	0	0	0	0	1	6
Seminal Vesicles								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	24	0	1	0	1	1	23
Coagulating Gland								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	24	0	1	0	1	1	23
Epididymides								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	24	0	1	0	1	1	22
Unilateral absence of spermatozoa	0	0	0	0	0	0	0	1
Testes								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	24	0	1	0	1	1	22
Unilateral atrophy	0	0	0	0	0	0	0	1
Pituitary								
Examined	0	24	0	1	0	1	1	23
No abnormalities detected	0	24	0	1	0	1	1	22
Cyst(s) in pars anterior	0	0	0	0	0	0	0	1
Factors Contributory To Death								
Examined	0	0	0	0	0	0	1	0
Poor clinical condition	0	0	0	0	0	0	1	0

TABLE 14

(Microscopic pathology - continued)

Females on study	Group 1 Control 24		Group 2 62.5 mg/kg/day 24		Group 3 250 mg/kg/day 24		Group 4 1000 mg/kg/day 24	
Animals completed	Decedent 1	Terminal 23	Decedent 1	Terminal 1	Decedent 0	Terminal 1	Decedent 2	Terminal 22
Uterus								
Examined	1	23	1	1	0	1	2	22
No abnormalities detected	1	17	0	1	0	1	2	18
Luminal dilatation (Total)	0	6	0	0	0	0	0	4
Minimal	0	6	0	0	0	0	0	4
Placentae present	0	0	1	0	0	0	0	0
Cervix								
Examined	1	23	1	1	0	1	2	22
No abnormalities detected	1	23	1	1	0	1	2	22
Vagina								
Examined	1	23	1	1	0	1	2	22
No abnormalities detected	1	23	1	1	0	1	1	22
Inflammatory exudate in lumen (Total)	0	0	0	0	0	0	1	0
Moderate	0	0	0	0	0	0	1	0
Ovaries								
Examined	1	23	1	1	0	1	2	22
No abnormalities detected	1	22	1	1	0	1	2	22
Bilateral follicular cysts	0	1	0	0	0	0	0	0
Pituitary								
Examined	1	23	1	1	0	1	2	22
No abnormalities detected	1	21	1	1	0	1	2	22
Cyst(s) in pars anterior	0	2	0	0	0	0	0	0
Oesophagus								
Examined	0	1	0	0	0	0	0	0
No abnormalities detected	0	1	0	0	0	0	0	0
Factors Contributory To Death								
Examined	1	0	1	0	0	0	2	0
Unknown	0	0	1	0	0	0	1	0
Intubation error	1	0	0	0	0	0	1	0

Conclusions

Remarks: “Based on the result obtained, this study indicated that dosages of 62.5, 250, or 1000 mg/kg/day were without adverse effect on the growth and reproductive capacity of male and female rats or the development of their offspring. The dosage of ODB-2 at which no signs of toxicity were recorded is therefore considered to be 1000 mg/kg/day.”

Data Quality

Remarks: None

References

Huntingdon Research Centre Ltd., “ODB-2: A Study in the Rat on Reproductive Function of One Generation by Oral Administation,” October 29, 1993.

Other

None